THE STUDY OF BREAD STALING USING VISIBLE AND

NEAR-INFRARED REFLECTANCE SPECTROSCOPY

by

FENG XIE

M.S., Zhengzhou Grain College, 1998

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A DISSERTATION

Submitted in partial fulfillment of the

requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry

College of Agriculture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2002

Approved by:

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ABSTRACT

The potential of visible and near-infrared reflectance spectroscopy (NIRS) as a fundamental tool for studying bread staling was investigated in this research. NIRS was used to detect changes in bread during storage and results were compared to those obtained by a texture analyzer (TA). NIR spectra had a high correlation to TA firmness. NIRS measurements correlated better with the actual storage time and had smaller standard deviations than the TA firmness. The batch differences had less effect on NIRS measurements than on the TA firmness. Results indicate that NIRS could monitor changes during bread storage more precisely than the TA. The potential of NIRS was further investigated by studying starch, protein, and temperature effects on bread staling with the help of a differential scanning calorimetry. Results show that NIRS could be a useful tool to study bread staling. Three important wavelengths, 970nm, 1155nm, and 1395nm, showed that NIRS monitored moisture and starch structure changes in bread staling. The other two important wavelengths, 550nm and 1465nm, successfully classified the starch-starch (SS) and starch-protein (SP) breads based on color and protein content differences in SS and SP. Results also show that amylopectin retrogradation was the major factor for bread staling. The amylose-lipid complex contributed little to bread staling after one day of storage. Temperature significantly accelerated the bread staling process. Protein retarded bread staling by diluting starch and reducing starch retrogradation. The effect of protein on bread staling was limited when compared to the temperature effect.

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1. INTRODUCTION

1.1 Overview

The mechanism of bread staling has been studied for 150 years, but is still of interest both scientifically and commercially. Much research has been conducted in this field, although results are not always consistent and the underlying mechanism is not yet fully understood. Currently, no single technique can provide a complete picture of all events related to staling. The reasons for this may be because bread staling is a complicated process and it is sensitive to flour types, additives, processing conditions, and storage conditions (Zobel and Kulp 1996).

Bread staling has commercial significance because bread is a staple food all over the world and its freshness is often lost quickly. For example in America, white pan bread is the principal bread product. However, bread usually has a short commercial shelf life because of bread staling. Product shelf life determines its distribution distance. For a wholesale distribution system, bread products having a shelf life of 5-days or more are required. Most white bread produced in America only has a 2-day shelf life even under optimum storage conditions (Maga 1975). Annually, approximately 20 billion pounds of bread are produced. About 3% (600 million pounds) of the products are returned and this is a considerable economic burden on both bakers and consumers (Zobel and Kulp 1996). Bakers who wish to extend their markets are motivated to enhance the shelf life of their product. Research that reveals more about the bread staling process and that reduces the staling rate would be significantly valuable for

them. In addition, an understanding of the mechanism of bread staling and technological knowledge to prevent or retard staling would be an asset to those involved in trying to alleviate world food problems. For example, the staple food in many developing countries is starchy foods prepared from cereals, roots, and tubers. Many of these foods also have a short shelf life due to staling. Starch retrogradation, one of the causes of bread staling, may play a central role in the shelf life of these foods as well (Kim and D'Appolonia 1977). In brief, the study of bread staling could lead to solutions that will bring great economic benefit to both of bakers and consumers in the future.

Various analytical techniques are available for investigating changes at macroscopic, microscopic, and molecular levels of bread staling, such as texture analysis, scanning electron microscopy, differential scanning calorimetry, and nuclear magnetic resonance (Schiraldi and Fessas 2001). Wilson et al (1991) successfully introduced near infrared reflectance spectroscopy (NIRS) to study bread staling in 1991. Osborne (1998) obtained similar results when following starch crystallinity in stored bread crumb using NIRS. NIRS is a simple, rapid, and non-invasive method. The results obtained by Wilson et al (1991) and Osborne (1998) indicated that NIRS could provide fundamental evidence for the mechanism of bread staling without any damage to the integrity of the bread. This is the advantage of using NIRS. It is imperative to consider the integrity of the bread system when studying bread staling because various events take place concurrently within the system. For example, when starch and protein are

analyzed separately, the integrity of the starch-protein system is overlooked. Currently, a less invasive approach in establishing the gluten and starch interaction is to measure the availability of starch in the system to amylolytic enzymes before and after incubation with proteases (Guerrieri et al 1997). A diode array visible and near infrared reflectance spectrometer was used in this study. Unlike Wilson et al (1991) and Osborne (1996) using the absorption value at a certain wavelength in the data analysis, the whole spectra were used in this study. Partial least square (PLS) regression and cross-validation were used to analyze NIR spectra data. These are all new to the study of bread staling, and may lead to a better understanding of the nature of bread staling.

Even though NIRS has been widely used to predict the chemical composition of many agricultural products, the theoretical basis of the technique is often neglected. The selected wavelengths are not always assigned to chemical groups (Millar et al 1996). Previous studies show that NIRS has the potential to be a fundamental tool for bread staling studies. To investigate this potential further will help scientists understand more about this technique, which will be helpful in developing NIRS applications further as a means for studying bread staling or other similar phenomenon.

1.2 Proposed Theories of Bread Staling Mechanisms

Bread staling includes physical and chemical changes in bread during storage, such as taste, aroma, firmness, opacity, crystallinity, crumbliness, water

absorptive capacity, susceptibility to attack by β–amylase, and crumb soluble starch content. (Knightly 1977; Ovadia 1994). Numerous studies have been conducted and several possible theories have been proposed. However, no clear-cut answer to the bread staling phenomenon has emerged yet.

1.2.1 Moisture Migration

Generally, the role of water in bread is to increase its shelf life. Increasing the moisture content of bread enhances softness and retards firmness. Generally, decreasing moisture content by 2% in the finished bread shortens the shelf life by one day (D'Appolonia and Morad 1981; Stauffer 2000). However, moisture migration accelerates bread staling (Willhoft 1971a,b; Piazza and Masi 1995; Zobel and Kulp 1996). Levine and Slade (1991) studied freezable water during storage in hermetically sealed bread samples using differencial scanning calorimetry (DSC). They found that the percentage of free water decreased from 21% on day 0 to 16% on day 11. In the meantime, starch crystallinity increased. The freezable free water migrated to the crystal region and became unfreezable. It was no longer available as a plasticizer of the gluten-starch network. As a result, the bread became firmer. The results demonstrate that moisture migration accelerates the bread staling process.

Water may either enhance the molecular mobility of polymer chains or act as a plasticizer between polymers in the bread staling process (Schiraldi and Fessas 2001). Schiraldi et al (1996) proposed a water migration model (Figure 1.1),

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which attributed bread firming to moisture migration. In this model, water molecules form a bridge between each-other-facing binding sites. A direct bond between chains can easily displace these bridge bindings. Then water molecules can diffuse to the next neighboring sites and promote the formation of a new direct inter-chain link along polymer chains. Water molecules, which act as sliders of an inter-chain zipper, promote an extension of cross-link networks throughout the bread crumb, such as starch crystallinity. As a result, water migration increases crumb firmness (Schiraldi et al 1996). Water binding compounds such as sugar, alcohol, pentosan, and hydrocolloid can reduce the bread firming rate. According to this model, those compounds compete with large biopolymers for water and reduce water activity, thus reducing water redistribution and starch retrogradation, and slowing the overall crumb firming.

Moisture migration has been a controversial subject. It was reported that retrograded starch absorbed the water released by transformed gluten during bread storage (Breaden and Willhoft 1971; Willhoft 1971a,b). Up to 30% moisture in the gluten fraction migrated to the starch during 120-hour storage at 25°C. The rate of migration decreased during storage. During baking, moisture migrated to starch at an accelerated rate (Willhoft 1971a). Others, such as Senti and Dimler (1960), suggested that water was expelled from the starch matrix because of retrogradation. Starch water sorbing capacity decreased rapidly with age while gluten sorbing capacity remained virtually constant. Senti and Dimler (1960) estimated that about 2% water in the starch fraction was lost during aging.

If gluten took up the lost moisture from starch, there would be a 12% gain in water in gluten. During bread staling, gluten rigidity changes little (Zobel and Kulp 1996). Therefore, the lost moisture is not taken up by gluten. Generally, the concept that moisture migrates from gluten to starch is accepted. However, in either case, the overall change in moisture of the starch portion during bread staling is small, approximately 2%. The extent of the moisture effect in bread staling is minimal compared with the overall increase in crumb firmness (Stauffer 2000). Boussingault (1852) found that bread firmed without moisture loss. If stored under the proper conditions, a five-day-old stale loaf gives a drier mouth feel than fresh bread, even though they have the same moisture content. Therefore, factors other than moisture migration must be involved in the bread staling process.

1.2.2 Starch Retrogradation

Katz (1928) concluded that starch retrogradation causes bread firming. Because of his work, numerous studies have concentrated on starch gelatinization and retrogradation (Willhoft 1973a; Kim and D'Appolonia 1977; Lineback 1984; Ovadia 1994; Cauvain and Young 1998).

In 1996, Zobel and Kulp (1996) proposed a model that attributed bread firming to starch (Figure 1.2). Many of the staling mechanisms proposed so far have been accounted for and integrated into this model to some extent. Starch has different physical states during the bread baking and aging stages. During baking, starch

granules are swollen and gelatinized. Crystallinity of branched amylopectin (AP) is disrupted, and some parts of the AP expand into the inter-granular space. At the same time, amorphous and single-helical amylose is released from starch granules. As the bread cools, amylose exists as retrograded double helix and forms juncture points that gel within the inter-granular space. It gives the initial loaf firmness to the fresh bread. During storage, the AP reforms into double helical structure and crystallizes again. It provides rigidity to both the swollen granules and the inter-granule materials. As a result, it firms bread crumb. Retrograded AP is more sensitive to heat than retrograded amylose. If bread is reheated, AP crystallinity is disrupted again and bread is re-freshened. In this model, gluten plays a minor role during firming because it is relatively inert to change with time.

Some research shows that other factors play a role in bread staling. Zobel and Senti (1959) reported that heat-stable bacterial α-amylase retarded the firming rate, but the starch crystallization rate in amylase-supplemented bread, measured by X-ray diffraction, was faster than that in control bread. Increased crystallinity alone was insufficient to produce a firmer crumb. The lack of firmness in bread of high starch crystallinity was due to amylase activity. Morgan et al (1997) studied the firming rate of starch bread and a normal standard wheat bread treated with an antistaling enzyme α-amylase. Starch retrogradation was measured by solid-state ^{13}C cross-polarization/magic-angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP/MAS NMR), which is a more accurate

method to measure starch retrogradation than X-ray diffraction. They found that the decreased starch retrogradation correlated well with decreased firming in treated starch bread. The results were different from those reported by Zobel and Senti (1959). Every et al (1998b) studied the firming rate of starch bread treated with α-amylase using ^{13}C CP/MAS NMR. The result shows that changes in firmness and double helical content are highly correlated.

Some researchers found that firmness and starch retrogradation, as measured by DSC, were parallel (Roulet et al 1988). The starch gel concentrations used in their study were 40% and 50%. However, Rogers et al (1988) studied AP re-crystallization using a DSC and found that the firming rate was faster than starch retrogradation rate when bread moisture content was 22%. This may be due to moisture content. Zeleznak and Hoseney (1986) studied starch gel by DSC and stated that water present during retrogradation controlled the magnitude of the amylopectin crystallinity. AP retrograded little in the diluted or concentrated gels. The greatest enthalpy, or the highest retrograded AP content, was obtained in the 50%~60% starch gels. Retrogradation was minimal in starch gels of less than 20% moisture but increased sharply between 20%~30%.

Generally, the fact that starch retrogradation is responsible for the bread staling is accepted. But some major points are not explained by this model, such as starch and protein changes during staling, and water redistribution.

1.2.3 Protein (gluten)

Researchers were aware that protein probably plays an essential role in bread firming since 1954 (Ovadia 1994). Later, a number of researchers studied the nature and significance of protein. Erlander and Erlander (1969) studied bread made from whole wheat flour and white wheat flour, which have different protein contents. The high protein bread was fresher than the low protein bread after 8 days storage. They concluded that protein inhibited starch retrogradation by forming a complex with starch, and the ratio of starch to protein in the dough was critical in determining the rate of staling. They suggested the amide group of glutamine protein interacted with a glucose unit in either the amylose or the amylopectin chain (Figure 1.3). But bread with high protein content tends to have high specific volume, which could affect these results. Maleki et al (1980) studied protein quality effects on bread staling. Flours were fractionated into gluten, starch, and water solubles. Original flours and reconstituted flours with interchanged fractions were used to make bread. They found that high protein content increases the loaf volume and results in softer bread. Gluten is regarded as the major fraction for differences in staling rate while starch and water solubles didn't significantly affect staling rate.

A model attributing bread firmness to starch-gluten interaction (Figure 1.4) was proposed by Martin and Hoseney (1991). In this model, the continuous gluten protein in the crumb is cross-linked (entanglements and /or hydrogen bonds) with the remnants of starch granules. The cross-linked network makes bread firm.

After starch gelatinizes, the partially soluble starch molecules and swollen granules may entangle with the gluten protein. During aging, the number and strength of the interaction between starch and gluten increase. The stiffened network makes bread firmer. Surfactants and starch fragments interfere with cross-linking and lead to softening.

The question if protein is a major contributor to bread staling has generated lively debate. Zobel and Kulp (1996) stated that starch gels underwent a rapid increase in rigidity during aging, and gluten firmed little. The solubility of protein was unaltered during bread staling while that of the carbohydrate components decreased during staling. The swollen granules were shrinking away from the gluten matrix during staling, leaving a channel between the two phases. Two separate physicochemical changes during staling were considered to occur: 1) a firming trend arising from starch retrogradation and 2) a relaxation of crumb structure detected as breaks in the firming curve. The latter could be explained as separation at the gluten/starch interface. Kim and D'Appolonia (1977) studied bread staling of different protein contents (10.6%~14%) at two storage temperatures. The resulting Avrami exponents were approximately 1.0. The Avrami exponents are characteristically related to the way that nucleation of crystallites occurs and to their subsequent growth. They concluded that the crystallization process was involved in bread staling, regardless of flour protein content, protein quality, and storage temperature. Proteins acted simply as diluents of starch and thereby reduced the firming rate. Every et al (1998a)

studied the firming rate of starch-starch and starch-protein bread. The starch-starch bread clearly increases in firmness up to six days. The bread of 10% and 15% gluten have similar specific loaf volume, moisture contents, and firming rates to those of the starch bread. They concluded that gluten plays a role in firming, including diluting the starch and reducing the firming rate. Gluten-starch and starch retrogradation are equally important in bread staling. Quantitatively, gluten-starch interaction is less important than starch-starch interaction because of the lower concentration of gluten.

1.2.4 Combination Models

Willhoft (1973b) proposed a "multi-component model" that attributed bread firming to starch, gluten, and moisture migration (Figure 1.5). During staling, moisture is released by gluten as part of the staling process. This makes gluten become more rigid. The released moisture is subsequently taken up through starch retrogradation. The overall crumb firmness is equal to the sum of the results of starch retrogradation, protein transformation, and softness of starch granules caused by the increase in its moisture level.

Every et al (1998b) proposed a starch and protein model that attributed bread staling to both starch and protein (Figure 1.6). During baking, glucan chains of amylopectin and amylose protrude from the starch granule. They cross-link with the amylose-amylopectin network via double helices to form an increasingly rigid crumb structure in the inter-granule space. During aging, glucan chains also

interact with protein fibrils. This model doesn't explain how gluten-starch interaction develops during staling.

It is clear that bread staling is a complex process. No single factor can explain the whole mechanism. Bread is a mixture of proteins (gluten), starch, and water in the approximate ratio of 1:6:5. Starch and proteins are in close contact in all steps of bread making. In bread, starch is embedded in a continuous three-dimensional gluten network and surrounded by continuous gas cells. Presumably staling is mainly due to changes occurring in the solid phase of crumb and is little influenced by the air cells (Fearn and Russell 1982). It may be reasonable to consider bread staling is caused by changes in starch, gluten, and moisture together. Combining the moisture migration model (Schiraldi et al 1996), the starch retrogradation model (Zobel and Kulp 1996), and the starch and protein model (Every et al 1998b) may be reasonable. Overall, starch, gluten, and moisture contribute to bread staling. Starch retrogradation is more important to bread staling than the gluten and starch interaction, quantitatively. Gluten affects bread staling by interacting with leached amylose and amylopectin via hydrogen bonds (cross-links) and entanglements. Moisture accelerates bread staling by increasing polymer transformations.

1.3 Applications of NIRS for Studying Bread Staling

NIRS has been widely used as a rapid analysis method for quality control. In the wheat milling industry, NIRS is applied to measure protein and moisture content

in both flour and kernels. It is also used to detect wheat and corn attributes such as class, color, damage, aflatoxin, and fumonisin (Dowell 2000). Suzuki et al (1986) applied NIRS to study bread constituents and quality parameters, such as moisture, protein, total sugar, and crude fat. Accuracy in determining moisture and protein was high whereas accuracy in sugar and lipid analyses was low. NIRS is also used to measure sucrose crystalline, starch crystalline, starch damage , and starch structure changes in starch-containing extruded products(Davies and Miller 1988; Millar et al 1996; Osborne and Douglas 1981).

Wilson et al (1991) first applied NIRS successfully to the study of bread staling. The staling rate measured by NIRS agreed with that obtained from DSC measurements. Osborne (1998) showed similar results when studying starch crystallinity in stored bread crumb using the wavelength range 1100-2500nm. When studying the bread staling phenomena, NIRS generally follows three physical and chemical changes occurring in bread crumb during storage: crumb scattering, moisture loss, and starch structure changes due to inter- and intra-molecule hydrogen bonds (Wilson et al 1991; Osborne 1996, 1998). The light scattering properties of the crumb is called crumb scattering.

Wilson et al (1991) reported that NIRS measured bread staling by following crumb scattering change. They found that the bread staling rate constant resulted from NIRS absorbance (log (1/R)) at wavelength 1934nm was consistent with that obtained by DSC. In order to test whether NIRS was following scattering

property change, spectra were subject to a multiplicative scatter correction and re-analyzed. Scattering correction can minimize most of but not all spectral differences due to physical properties of the sample. After scattering correction, log (1/R) at 1934nm showed no correlation with storage time but a high correlation with moisture content. The results demonstrated that NIRS spectra provided not only information about bread crumb light scattering property changes but also about moisture loss during storage. Wilson et al (1991) also found that log (1/R) obtained on day 1 were generally lower than what was obtained on day 14 over the whole wavelength range. This indicates an increase in light scattering, which was due to starch crystallinity development in staling. NIRS detects kernel hardness and vitreousness by following their effect on light scattering (Delwiche 1993; Dowell 2000). It is reasonable for NIRS to follow bread staling in an analogous manner because starch crystallinity changes crumb physical properties such as light scattering.

In addition, Wilson et al (1991) and Osborne (1996) reported that NIRS provided information about starch structure changes due to inter- and intra-molecule hydrogen bonds. Wilson et al (1991) noted that the staling rate constants calculated from the second derivatives of log (1/R) at wavelengths 1414 (or 1412) and 1465 (or 1466) nm were similar to those obtained from DSC measurements. Iwamoto et al (1986) had previously assigned these wavelengths to different hydrogen-bond states of water in food such as intra- and inter-molecular hydrogen bonds. Absorption at wavelengths 1412, 1466, and 1510nm

have been assigned to OH in water with no hydrogen bond (W_0), with one hydrogen bond (W_1), and with two hydrogen bonds (W_2). Absorption at wavelength 1430 and 1520nm is associated with OH in starch with no hydrogen bond (S_0) or with one hydrogen bond (S_1), which could be inter- or intra-molecular to water molecules (Osborne and Douglas 1981; Davies and Miller 1988; Osborne 1996). Osborne (1996) reported that W_0 decreases, while W_1 and W_2/ S_1 increased, due to starch crystallinity development in staling. A β-type crystalline region is commonly observed in bread crumb and caused by double helix amylose chains and amylopectin side chains. At saturation, β-type crystalline starch has 27% moisture (w/w). As a result, crystalline amylopectin must incorporate water molecules while starch chain segments realign (Zobel and Kulp 1996). Starch crystals become extensively hydrogen bonded, both intra-molecularly and inter-molecularly to water molecules. Osborne (1996) concluded that NIRS spectra over the range 1350-1650nm provided information about changes in starch structure during bread staling. NIRS could measure bread staling by following the changes of hydrogen bonds in the crystalline starch network (Wilson et al 1991; Osborne 1996, 1998).

Other related research also confirmed that NIRS could follow starch structure changes. Davies and Miller (1988) reported that crystalline sucrose had a characteristic absorption band at 1440nm because of a free hydroxyl group on the C_4OH bond. They noted that the absorption due to the first overtone of the OH stretching vibration is generally associated with the 1400-1500nm region in

NIRS spectra. Osborne and Douglas (1981) obtained a high correlation between NIRS measurements and starch damage using a calibration made from four wavelengths, 1442, 1580, 2060, and 2258nm. The wavelengths 1440, 1528, and 1588nm were assigned to the first overtone of the starch OH stretching vibration in the free, intra-, and inter-molecule hydrogen bond. Millar et al (1996) reported that absorption at 1520 and 1587nm decreased and absorption at 1428 shifted towards longer wavelength as starch crystallinity degree decreased in extruded products. Changes in these wavelengths were related to intra- and inter-molecular hydrogen bond of the starch. The density of hydrogen bonds in the system could cause the absorption wavelength shift.

1.4 Objectives

The overall objective of this research was to investigate the potential of NIRS as a fundamental tool for studying bread staling. The first specific objective was to investigate how well visible and NIRS measures bread changes during storage and to compare the NIRS method to the TA method. The second objective was to investigate starch, protein, and temperature effects on bread staling using NIRS and DSC.

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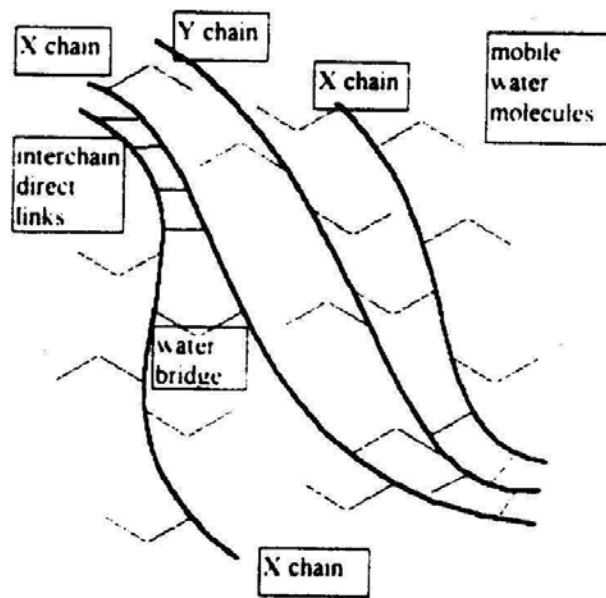
Figure 1.1 Moisture migration model (Schiraldi et al 1996).
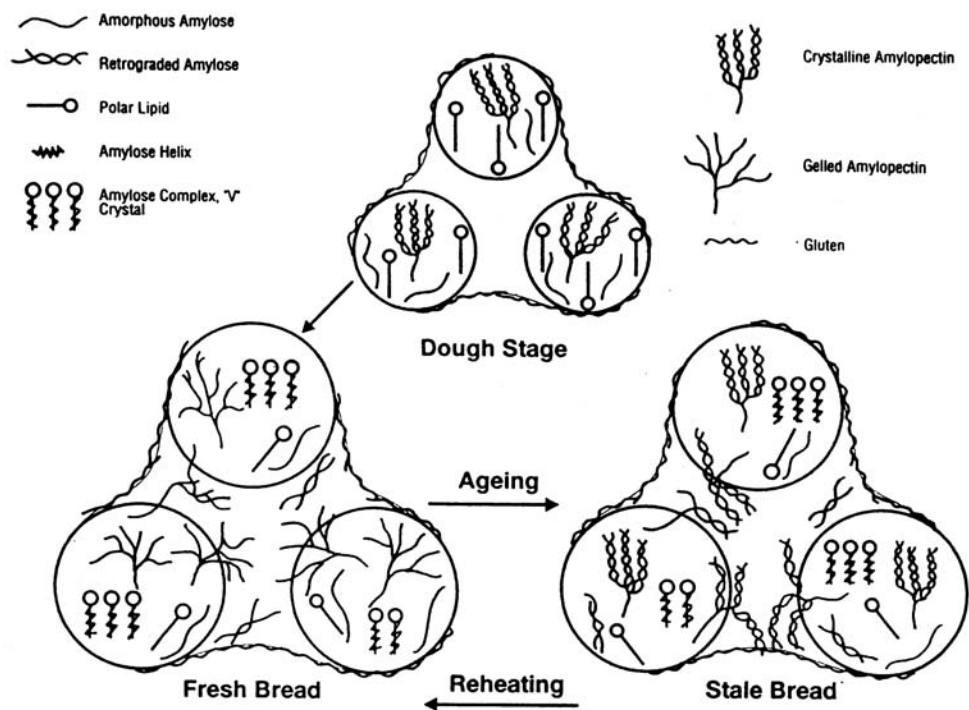
Figure 1.2 Starch retrogradation model (Zobel and Kulp 1996).
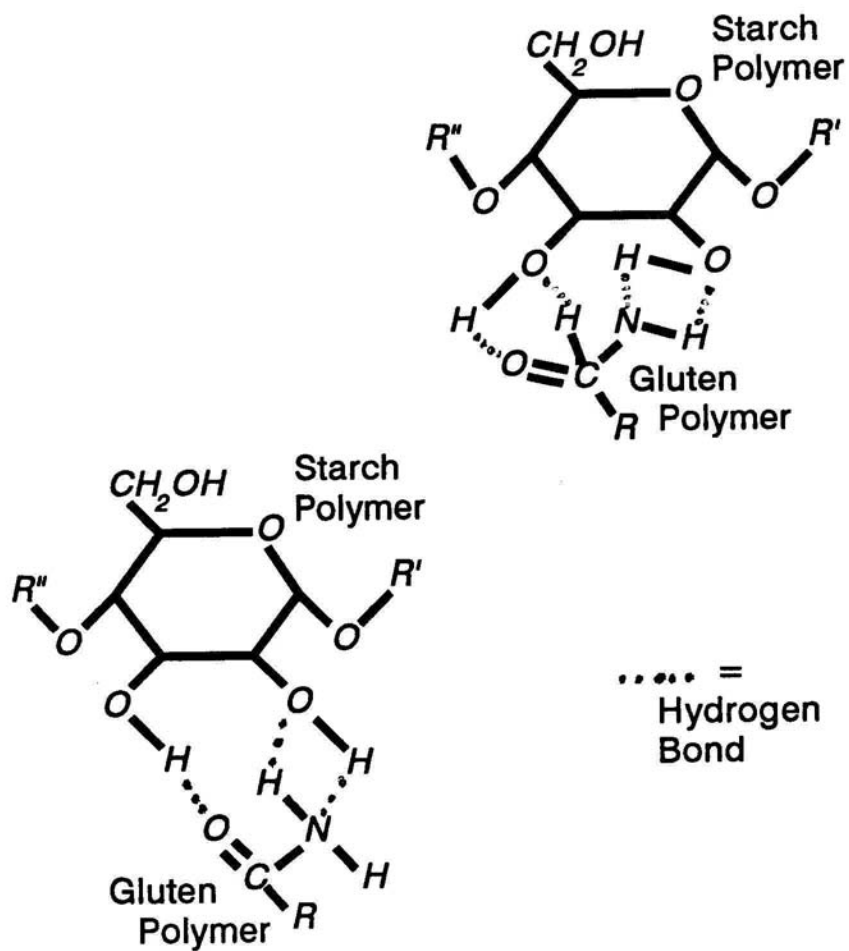
Figure 1.3 Hydrogen bond between gluten and starch

(Erlander and Erlander 1969).
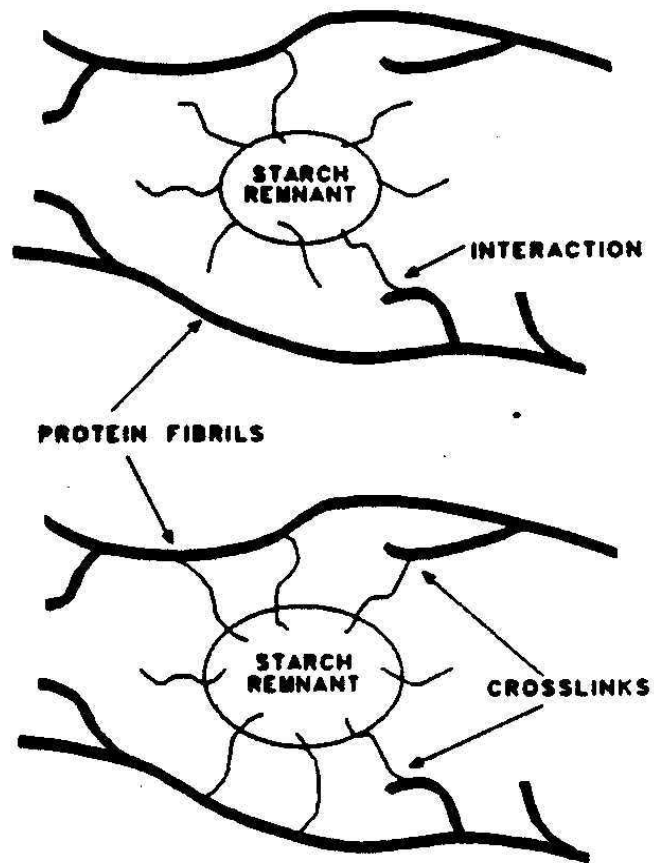
Figure 1.4 Gluten-starch interaction model (Martin and Hoseney 1991).
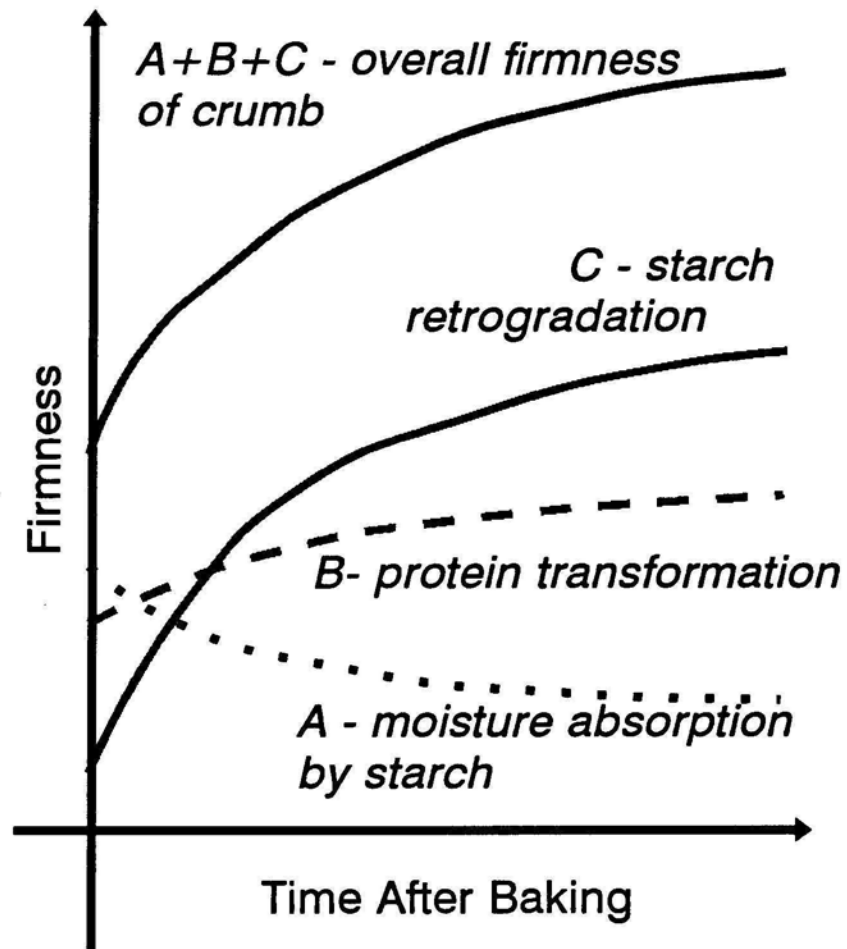
Figure 1.5 Multi-component model (Willhoft 1973b).
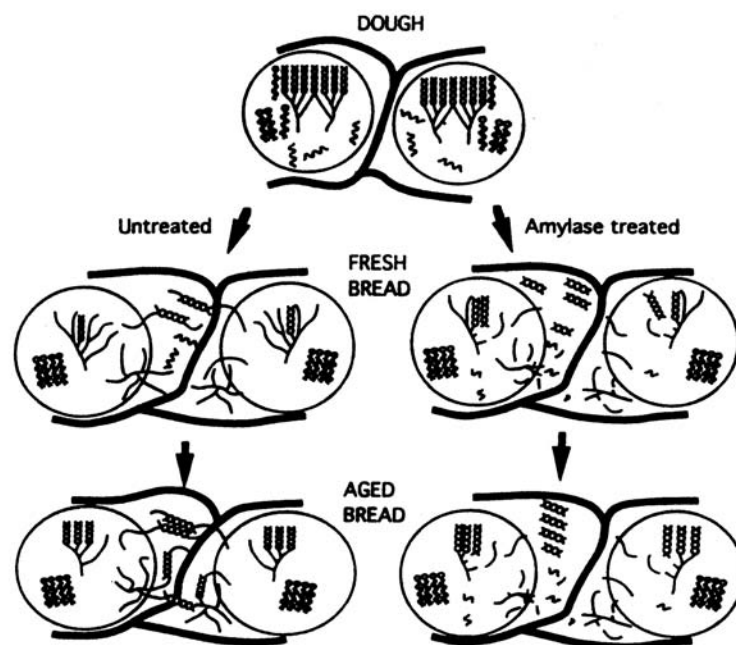
Figure 4. Model for bread staling and the anti-staling effect
of bacterial alpha-amylase

Protein network		Dextrins
Double helix of glucan chains; detected by NMR; not detected by X-Ray in non-crystalline regions		
Amylose-lipid complex; detected by NMR; not detected by X-Ray in non-crystalline regions		
Lipid-free amylose, possibly in a randome single-helix; not detectable in moist starch granule by NMR or X-Ray		
Crystalline amylopectin; detectable by NMR and X-Ray		
Amorphous amylopectin; not detectable by NMR or X-Ray		
Crystalline amylose-lipid conformation; detectable by NMR and X-Ray		
Amylose & amylopectin fragments in double helix and crystalline conformation		

Figure 1.6 Starch and gluten model (Every et al 1998b).

2. COMPARISON OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY AND A TEXTURE ANALYZER FOR MEASURING WHEAT BREAD CHANGES IN STORAGE*

2.1 Abstract

Bread staling affects bread texture properties and is one of the most common problems in bread storage. Bread firmness, as measured in compression mode by a texture analyzer (TA), has been commonly used to measure bread staling. This study investigated the potential of visible and near-infrared reflectance spectroscopy (NIRS) to detect bread changes during storage by comparing NIRS results with those obtained by the TA. Twenty-five loaves of commercial wheat white pan bread from one batch were studied over 5 days. NIRS and TA measurements were made on the same slice at approximately the same time. The experiment was repeated 5 times using the same kind of commercial samples from 5 different batches. NIRS measurements of slices, loaf averages, and daily averages were compared with TA measurements. NIRS spectra had a high correlation to TA firmness. NIRS measurements correlated better with the actual storage time and had smaller standard deviations than the TA measurements. The batch differences had less effect on NIRS measurements than on the TA measurements. The results indicate that NIRS could follow bread

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changes during storage better than TA. NIRS is probably based on both physical and chemical changes during bread staling, unlike the TA method that only measures bread firmness, which is only one aspect of the staling phenomenon.

2.2 Introduction

Bread staling is one of the most common problems in bread storage. Bread firmness correlates negatively with the organoleptic assessment of staleness (Bice and Geddes 1949). As a result, firmness becomes an important attribute in assessing bread staling. Current standard methods of testing firmness include the universal testing machine (UTM) (AACC 74-09) and the baker compressimeter (BC) (AACC 74-10). The BC method was recommended by the AACC in 1947. Use of the BC is a valid method for measuring firmness but can only detect limited physical properties. This drawback limits the BC's applications (Kamel et al 1984). Researchers began to use the UTM and texture analyzer (TA) methods in the 1980's. Even though the TA has not been officially adopted as the standard AACC method of testing, the TA is commonly used and AACC method 74-09 is applicable to the TA (Hebeda and Zobel 1996). The American Institute of Baking (AIB) standard procedure for white pan bread is designed specifically for testing white pan bread firmness with the TA-XT2 texture analyzer. Since white pan bread was chosen for this study, firmness was measured by using the TA.

Visible and near-infrared spectroscopy (NIRS) is widely used in the wheat milling industry for measuring protein and moisture content. NIRS has also been used to detect wheat and corn attributes such as class, color, damage, aflatoxin, and fumonisin (Dowell et al 2002). Suzuki et al (1986) applied NIRS to study bread constituents and quality parameters such as moisture, protein, total sugar, and

crude fat. NIRS applications have typically been directed at rapid analysis for quality control.

NIRS was first used by Wilson et al (1991) to study bread staling. Osborne (1998) studied starch crystallinity in stored bread crumbs using NIRS. The wavelength range used in both studies was 1100-2500 nm. Osborne (1996) investigated starch and water in bread using the NIRS wavelength range of 400 to 2498 nm.

Generally, results from these studies showed that three changes could be followed by NIRS. These include crumb scattering, moisture loss, and starch structure changes due to inter- and intra-molecule hydrogen bonding. Crumb scattering refers to the light scattering properties of the crumb. It was reported that the bread staling rate constant calculated from NIRS absorbance (log (1/R)) at wavelength 1934 nm was in close agreement with that obtained by differential scanning calorimetry (DSC). After scattering correction, absorbance at 1934 nm had no correlation with storage time, but a high correlation with moisture content (Wilson et al 1991; Osborne 1998). This demonstrated that NIRS could provide information about scattering change and moisture loss during storage. It was found that absorbance generally decreased over the whole range as crystallinity developed during bread staling. This may be due to starch crystallinity development in bread (Wilson et al 1991; Osborne 1998). NIRS detects kernel texture (hardness) by virtue of its effect on scattering. It is reasonable for NIRS to

follow bread staling in an analogous manner because starch crystallinity changes crumb physical properties such as scattering of NIR radiation.

In addition, it was observed that the rate constants calculated from the second derivatives of log (1/R) at wavelengths 1414 (or 1412) and 1465 (or 1466) nm were similar to those obtained from DSC measurements (Wilson et al 1991; Osborne 1998). Iwamoto et al (1986) had previously assigned these two wavelengths to different hydrogen-bond states of water in food such as intra- and inter-molecular hydrogen bonds. Osborne (1996) stated that spectra over the range 1350-1650 nm provide information about changes in starch structure during bread staling. Absorption at wavelengths 1412, 1466 and 1510 nm has been assigned to OH in water with no hydrogen bond (W_0), with one hydrogen bond (W_1), and with two hydrogen bonds (W_2), respectively. Absorptions at wavelengths 1430 and 1520 nm are associated with OH in starch with no hydrogen bond (S_0) or with one hydrogen bond (S_1), which could be intra- or inter-molecular to adjacent water molecules (Davies and Miller 1988; Osborne and Douglas 1981; Osborne 1996). Osborne (1996) reported that W_0 decreased, while W_1 and W_2/S_1 increased during storage due to starch crystallinity development. Starch crystallinity is extensively hydrogen bonded, both intra-molecularly and inter-molecularly to solvent water. These results demonstrated that NIRS could measure bread staling by following the changes of hydrogen bonding in the starch crystallinity network (Wilson et al 1991; Osborne 1996, 1998).

Previous research showed that NIRS has the potential to measure bread staling. However, no information has been available about how well NIRS can measure bread staling. The objective of this study was to investigate how well NIRS measures bread changes in storage and compare the NIRS method with the TA method.

2.3 Materials and Methods

2.3.1 Bread samples

Bread samples (Dillon signature homelike white bread of brand name: "Dillons") were purchased from a local grocery store. Bread was baked from frozen dough in the store. The baker was instructed to cut each loaf into 12.5 mm slices after cooling down, and wrap the loaf in a single plastic bag. Twenty-five loaves (16 oz per loaf) were obtained from each batch. Five fresh loaves were tested on the day of purchasing, which was named as day 1. The others were stored in a chamber at temperature ($27^{o}C \pm 1^{o}C$) and humidity ($50\% \pm 1\%$) for 1, 2, 3, and 4 days. Five loaves were taken out each day about 30 min before measurement. The two or three heel slices of each loaf were excluded. The experiment was repeated 5 times using bread from 5 different batches, referred to as batch *a*, *b*, *c*, *d*, and *e*.

2.3.2 NIR spectra collection

A diode-array NIR spectrometer (DA7000 Perten Instruments, Springfield, IL) was used to collect spectra. The wavelength range was from 400 nm to 1700 nm.

Data were recorded as log (1/R), where R is the relative reflectance. A reference standard Spectralon® was used to collect the baseline. Each spectrum was recorded as an average of 15 scans taken in about one second.

In each loaf, measurement was taken on each of five or six sets of two adjacent slices at room temperature and humidity. The bag was re-closed after every two adjacent slices were taken out. During scanning, the two adjacent slices were placed on top of each other on a horizontal flat glass window (diameter 12.7cm); and the orientation was kept constant for all tests. The light source illuminated the sample through the window, then the reflected light went back though the window and to the detector. After NIRS scanning, the slices were immediately measured using the TA test.

2.3.3 Texture analyzer data collection

A TA-XT2 (Texture Tech. Corp., Scarsdale, NY) was used to measure bread firmness by following the AIB standard procedure for white pan bread. A TA-3 1" acrylic cylinder probe was used according to the procedure. The probe compressed the sample by 6.2 mm at a pre-test, test, and post-test speed of 2.0, 1.7, and 10.0 mm/second, respectively. The compression force was 10 gram. The maximum peak force in compression was recorded as the firmness value in gram units. The TA and NIRS measurements were taken from the same side of each two adjacent slices at approximately the same time. The sample orientation was kept constant in all TA tests. Only one measurement was taken from the

center of each of the two adjacent slices. About five or six measurements were taken from each loaf. The samples were discarded after the TA test.

2.3.4 Data analysis

Grams/32 software (Galactic, Salem, NH) and a partial least squares (PLS) regression (Martens 1989) were used to analyze NIR spectra data. Due to a low sensitivity of the sensor and intensity of the light source, spectra have a high noise level below a wavelength of 550 nm. Therefore, the wavelength range from 550 to 1700nm was used in data analysis. Beta coefficients were used to determine the important wavelength regions for calibration models. Experimental data from the NIRS and TA measurements were calculated separately. For the TA data, the original data obtained from one loaf were averaged and recorded as the loaf average. The loaf daily averages in the same test were averaged and recorded as the daily average. For the NIRS data, the loaf and daily averages were calculated from values obtained from the cross-validation and from independent samples that were not included in the calibration.

Cross-validation was applied to optimize calibration models and detect outliers. When doing a cross-validation, one sample was removed from the data set, and then a calibration was made using the rest of the samples. The removed sample was measured by the calibration. All samples in the data set would be left out and measured once in turn. The value of residual error sum of squares (PRESS) showed the effect of adding a PLS factor to the calibration model. A calibration

was selected based on the PRESS value. The other method of developing a calibration was to combine all samples from a few batches. The rest of the batches were considered as unknown samples and measured by the calibration model. The results were called NIRS measurements in this study.

According to the AIB procedure, the bread staling trend was investigated by studying the relationship between the TA measurements and sample's actual storage time. In order to compare the NIRS and TA methods, NIRS spectra were correlated with actual storage time. The bread staling trend was studied by plotting NIRS measured storage time against the actual storage time for the NIRS method. For certain bread samples stored under certain conditions, storage time can be used to indicate the sample's staling level.

2.4 Results and Discussion

The relationship between NIRS spectra and TA firmness was studied. A calibration was made from batches *a* and *e* because they had the highest and lowest R^2, respectively, on cross-validation samples. Samples in batches *b*, *c*, and d were measured by using this calibration. The results are shown in Figure 2.1. Standard error of estimation was 47.44 and R^2 was 0.79. The results indicate that NIRS could follow firmness changes occurring during bread staling.

When studying the bread staling trend, the results of the NIRS and TA measurements were compared among different batches and within each batch.

Summarized results are shown in Table 2.1. The average R^2 of 5 batches obtained by the TA was 0.34, 0.43, and 0.84 for slices, loaf averages, and daily averages, respectively. The average R^2 obtained by the NIRS cross-validation for slices, loaf averages, and daily averages was 0.92, 0.96, and 0.99, respectively. The higher R^2 obtained by the NIRS indicates that higher proportion of total variability in NIRS spectra can be accounted for by samples' actual storage time. . Comparisons within each batch (Table 2.1) also show that the NIRS cross-validation had higher correlation with the actual storage time.

Figures 2.2 and 2.3 show the results of TA measurements and the NIRS cross-validations obtained from batch *c*. For each method, the bread staling trend was shown by plotting slices, loaf averages, and daily averages against actual storage time. For all slices, loaf averages, or daily averages, the NIRS cross-validation correlated better with actual storage time than TA measurements. The range of R^2 obtained by the NIRS from batch c was from 0.97 to 1.00. The range of R^2 obtained by the TA was from 0.69 to 0.98. Also, the NIRS cross-validation had smaller standard deviation (SD) than TA measurements for both loaf averages and daily averages. According to the AIB procedure, the daily average and its SD were recorded as the final bread firmness and SD. For daily averages, the NIRS method had larger R^2 and smaller SD than the TA method. This indicates that the NIRS cross-validation correlated better with actual storage time in batch *c* than TA measurements. The smaller SD shows that the NIRS was more precise in indicating staling level. In addition, TA measurements had more

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overlapping among samples of different actual storage times than NIRS cross-validations (Figures 2.2 and 2.3). The results indicate that the TA had more difficulties when classifying bread staling levels than the NIRS method.

TA measurement (daily averages) vs. storage time in different batches is shown in Figure 2.4. The relationship of daily averages for NIRS cross-validation vs. actual storage time in different batches is shown in Figure 2.5. Apart from a higher R^2, NIRS cross-validation had smaller SD and less overlapping among samples of different storage times than TA measurements. This shows that the precision of the TA for measuring bread staling levels is lower than the NIRS method. In addition, the NIRS cross-validation regression lines were more parallel and closer to each other than were the TA lines. This demonstrates that batch differences have less effect on the NIRS cross-validation than on TA measurements. Therefore, NIRS measures the actual staling level more precisely than TA.

Figure 2.4 shows that the regression lines for batches *b* and *c* had the highest and lowest line slops.   A calibration model was developed for the NIRS method by combining samples from batches *b* and *c*. The model was used to predict the storage time of samples from batches *a*, *d*, and *e*. The results are given in Table 2.1. For all slices and loaf averages in each batch, NIRS measurements had slightly lower R^2 value when compared to the NIRS cross-validation results. For NIRS daily averages, the R^2 was 0.88, 0.83, and 0.99 for batches *a*, *d*, and *e*,

respectively. For the TA daily averages, the corresponding R^2 was 0.85, 0.88, and 0.56, separately. The average R^2 of the NIRS for slices, loaf averages, and daily averages was higher than that of the TA. Daily averages of NIRS measured values in *a*, *d*, and *e* batches were plotted against actual storage time in Figure 2.6. The overall R^2 for the three batches was 0.66 and SEE was 0.70. For any unknown loaf, the 95% confidence interval of the prediction by using this calibration was 0.85 days. The results show that this calibration model could precisely measure actual bread staling levels among samples made from different batches.

Bread firmness is a partial indicator of bread texture. This gives a useful means for routine assessment of bread, but doesn't give a fundamental measure of staleness (Fearn and Russell 1982). NIRS not only follows physical changes in bread staling, but also provides chemical information without any damage to the integrity of bread. Physical changes refer to scattering property changes, which are modified by developed crystallinity during bread aging. NIRS can also reflect chemical information, such as moisture loss and starch structure changes during staling (Wilson 1991; Osborne 1996, 1998). This is likely the reason that the NIRS method showed superior testing precision when measuring bread staling level. Moisture loss and starch crystallinity change could cause firmness development during bread storage time. This can also explain that why NIRS could follow bread firmness changes during storage.

It was noticed that batch *d* and *e* had a smaller bread volume than the other batches. The volume differences among batches may have affected TA measurements and decreased the precision of this method. However, the volume differences had less effect on NIRS measurements. Fern and Russell (1982) reported that high specific volume loaves would have a lower initial crumb modulus that indicated softer texture, and showed a smaller change in modulus with time on staling than those of low specific volume. Another observation was that no trend existed in NIRS measurements throughout a loaf. Many researchers have found that the center slices of the loaf tended to be firmer than the outer slices (Short and Roberts 1971; Redlinger et al 1985). The original TA measurement did vary throughout the loaf, with the firmest crumbs at the center of the loaf. The reason why this firmness pattern exists is not clear and may due to lower specific volume, moisture migration, less gelatinized starch, and larger starch crystals in the center (Short and Roberts 1971; Piazza and Masi 1995). Based on these two observations, future work should study specific volume effects on NIRS measurements. Even though NIRS could measure bread changes in storage well, the theoretical basis of the technique is not clear yet. Further investigation of this NIRS method will be helpful for developing this technique. Research is needed to interpret beta coefficients for NIRS spectra to assign a wavelength to specific chemical changes that influence bread staling.

2.5 Conclusion

NIRS spectra had a high correlation to TA firmness. NIRS measurements had a higher correlation to bread storage time than the TA measurements. This

indicates that the NIRS measures bread changes more precisely than the TA. Batch differences had less effect on NIRS measurements. This shows that the NIRS model could measure changes in bread samples made from different batches more precisely than the TA method. The NIRS method is based on both physical and chemical changes during bread staling, unlike the TA method that only measures bread firmness, which is only one aspect of the staling phenomenon. Future work should investigate specific volume effects on NIRS measurements and interpret beta coefficients for NIRS spectra.

2.6 Acknowledgments

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Table 2.1 Summary of the NIRS and TA comparison.

| Batches | | TA | | NIRS | | | |
| | | | | cross-validation | | validation[a] | |
		$R^{2\,b}$	SEE[c]	R^2	SEE[d]	R^2	SEE[d]
a	Slice	0.27	64.89	0.84	0.53	0.56	0.81
	Loaf	0.37	53.67	0.91	0.41	0.62	0.74
	Daily	0.85	21.44	0.97	0.30	0.88	0.43
b	Slice	0.07	63.85	0.95	0.32	~	~
	Loaf	0.14	45.64	0.98	0.19	~	~
	Daily	0.90	7.57	1.00	0.11	~	~
c	Slice	0.69	49.06	0.97	0.23	~	~
	Loaf	0.83	34.06	0.99	0.16	~	~
	Daily	0.99	9.64	1.00	0.09	~	~
d	Slice	0.43	74.21	0.91	0.42	0.68	0.76
	Loaf	0.48	69.57	0.96	0.28	0.83	0.65
	Daily	0.88	31.12	0.98	0.22	0.83	0.63
e	Slice	0.24	70.84	0.95	0.31	0.79	0.38
	Loaf	0.33	59.40	0.97	0.21	0.92	0.22
	Daily	0.56	45.32	0.99	0.14	0.99	0.10
Average	Slice	0.34	64.57	0.92	0.36	0.68	0.65
	Loaf	0.43	52.47	0.96	0.25	0.79	0.54
	Daily	0.84	23.02	0.99	0.17	0.90	0.39

[a] A calibration was developed from batch *b* and *c*, and used to predict samples of batches *a*, *d*, and *e*.
[b] All correlations are with actual storage time.
[c] The TA SEE refers to standard error of estimation in gram (force) unit.
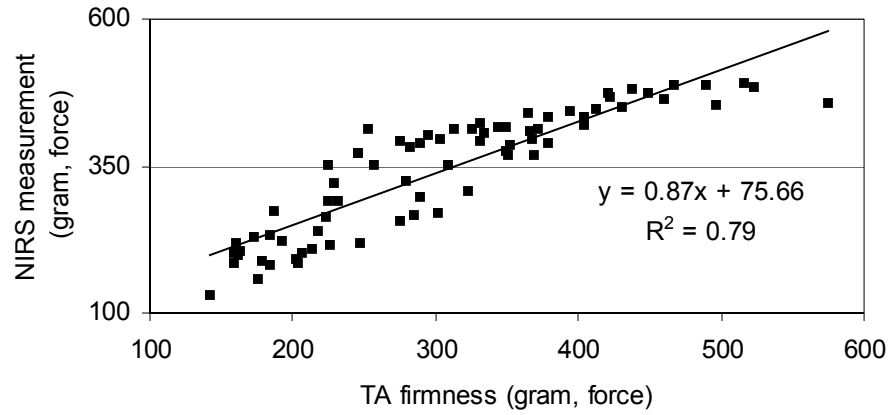[d] The NIRS SEE refers to standard error of estimation in day units.

Figure 2.1 Correlation of firmness measured by NIRS (8 factors) to firmness measured by TA.
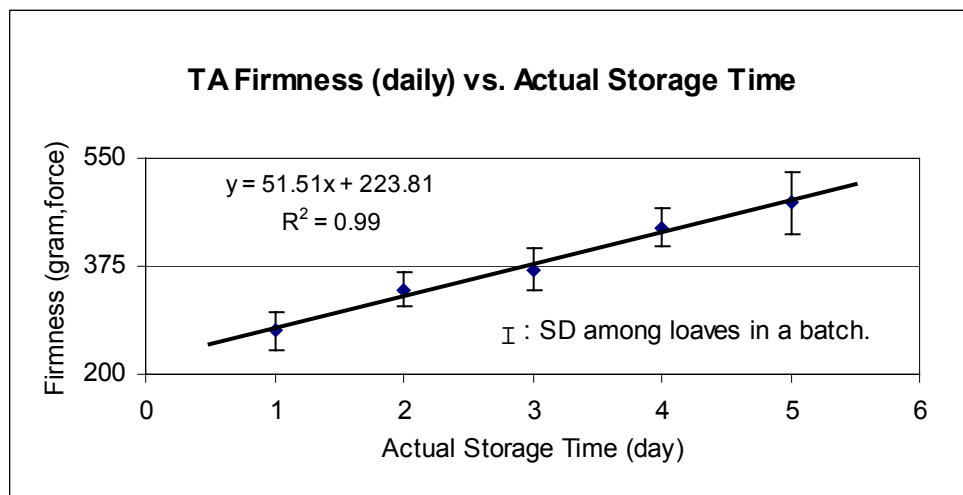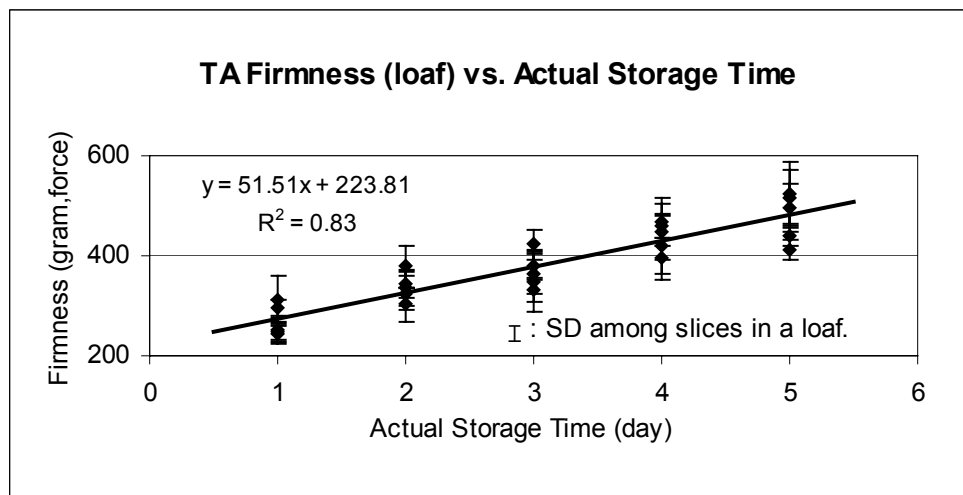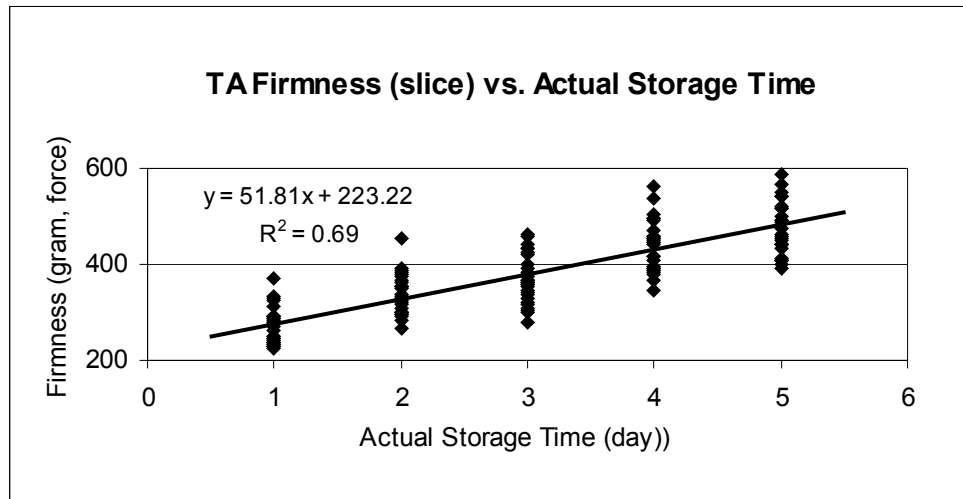
Figure 2.2 Correlation of TA firmness with actual storage time in batch *c*.
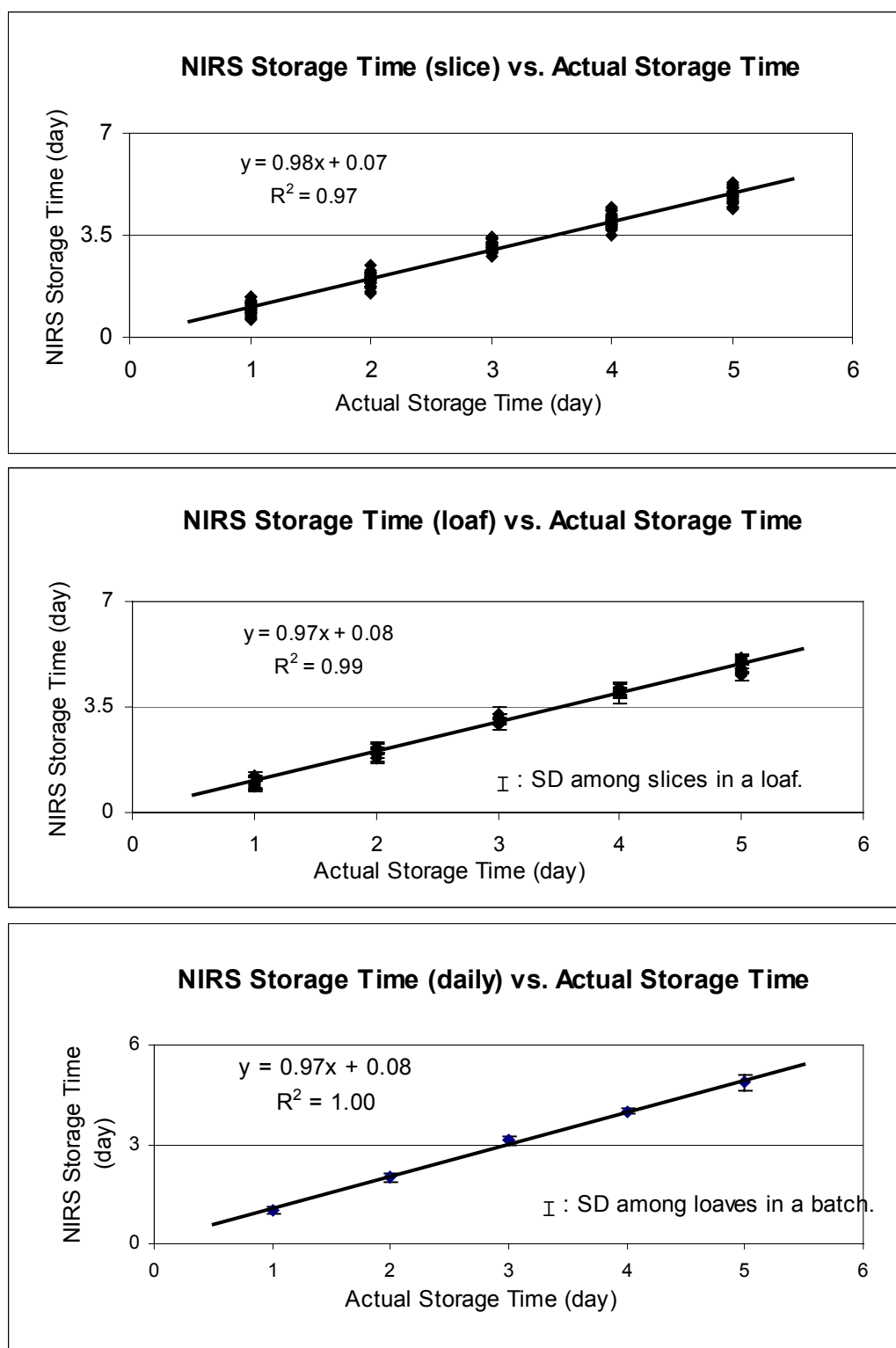
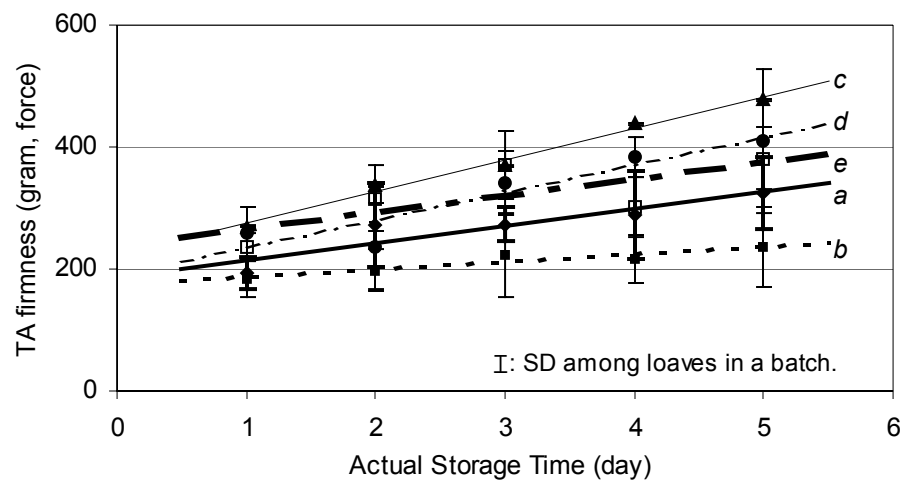**Figure 2.3 NIRS cross-validation results in batch *c*.**

Figure 2.4 Actual storage time vs. TA firmness (daily) for five batches.
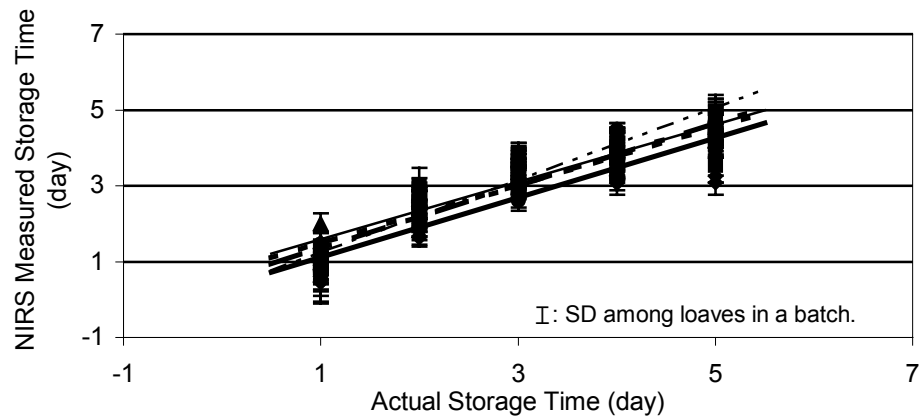
Figure 2.5 NIRS results (daily) in five batches using cross-validation method (legends as in Figure 2.4).

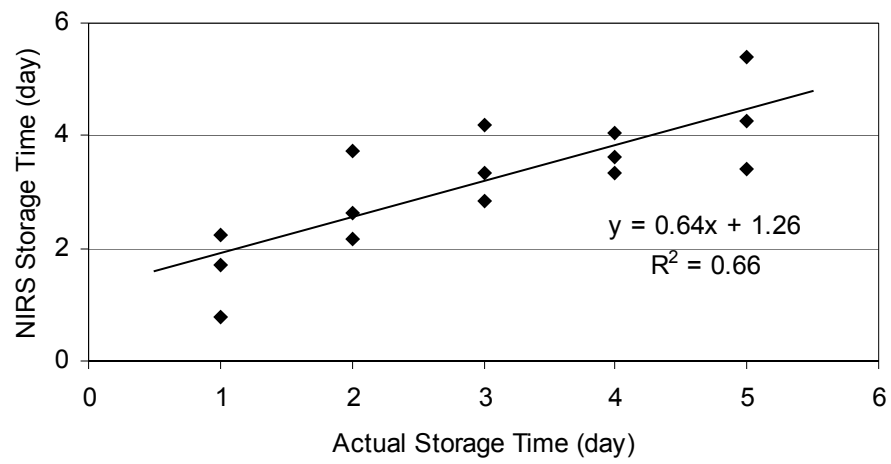$$y = 0.64x + 1.26$$
$$R^2 = 0.66$$

Figure 2.6 Correlation of storage time predicted by NIRS (8 factors) to actual storage time.

3. Using Visible and Near-Infrared Reflectance Spectroscopy and Differential Scanning Calorimetry to Study Starch, Protein, and Temperature Effects on Bread Staling

3.1 Abstract

Starch, protein, and temperature effects on bread staling were investigated using visible and near-infrared spectroscopy (NIRS) and differential scanning calorimetry (DSC). Bread staling was mainly due to amylopectin retrogradation. NIRS measured amylopectin retrogradation precisely in different batches. Three important wavelengths, 970nm, 1155nm, and 1395nm, were associated with amylopectin retrogradation. NIRS followed moisture and starch structure changes when amylopectin retrograded. The amylose-lipid complex changed little one day after baking. The capability of NIRS to measure changes in the retrograded amylose-lipid complex was limited. Two important wavelengths, 550nm and 1465nm, were key for NIRS to successfully classify the starch-starch (SS) and starch-protein (SP) bread based on different colors and protein contents in SS and SP. Low temperature dramatically accelerated the amylopectin retrogradation process. Protein retarded bread staling, but not as much as temperature. The starch and protein interaction was less important than the starch retrogradation. Protein hindered the bread staling process mainly by diluting starch and retarding starch retrogradation.

Key words: bread, staling, near-infrared, spectroscopy, protein, starch

3.2 Introduction

Bread staling is a complex process that occurs during bread storage. The mechanism of bread staling is still not clear yet even though it has been studied for 150 years. Generally, the fact that starch retrogadation is responsible for bread staling is accepted. Starch retrogradation is mainly due to amylopectin retrogradation. Amylose contributes to bread staling primarily in the first 24 hours after baking (Kim and D'Appolonia 1977b; Zobel and Kulp 1996). Previous research on bread staling, however, has not always produced consistent results and the underlying mechanism is not fully understood. For instance, whether protein is a major contributor to bread staling has generated lively debate (Erlander and Erlander 1969; Kim and D'Appolonia 1977a; Maleki et al 1980; Martin and Hoseney 1991; Every et al 1998). Currently, no single technique has provided a complete picture of all events related to this process.

Suzuki et al (1986) used visible and near-infrared reflectance spectroscopy (NIRS) to study bread constituent content. They reported that moisture and protein could be precisely determined whereas sugar and lipid analyses were less accurate. Wilson et al (1991) applied NIRS to study the bread staling phenomenon. The staling rate measured by NIRS agreed with that obtained from differential scanning calorimetry (DSC) measurements. Osborne (1998) showed similar results when studying starch crystallinity in stored bread crumbs using NIRS. Xie et al (in press) compared the NIRS method with the texture analysis (TA) method when assessing wheat bread changes in storage. Their results

indicated that the NIRS method was superior to the TA method for measuring bread staling during storage. NIRS followed the changes in bread physical state that caused the light scattering properties to change, and also followed specific functional molecular group changes, such as hydrogen bond changes associated with O-H bonds in water and starch (Wilson et al 1991; Osborne 1996, 1998). Others have reported that NIRS successfully measured starch crystalline, sucrose crystalline, and starch damage by following hydrogen bonds in water and starch (Osborne and Douglas 1981; Davies and Miller 1988; Millar et al 1996). Therefore, NIRS has the potential to provide the fundamental evidence for determining the mechanism of bread staling.

DSC is also a useful tool for studying bread staling because it measures starch retrogradation accurately and starch retrogradation largely accounts for bread staling (Fearn and Russell 1982; Wilson et al 1991; Osborne 1998). When a staled bread sample is heated in a DSC, an endotherm is observed when the crystallized starch melts again. Amylopectin endothermic peak temperature ranges from $50^{o}C \sim 80^{o}C$, depending on storage temperature, starch concentration, and storage time. Below $100^{o}C$, no endothermic peak is associated with the amylose fraction (Siljestrom et al 1988; Wilson et al 1991; Zobel and Kulp 1996; Klucinec and Thompson 1999). An endothermic peak at about $100^{o}C$ may correspond to the transition of the amylose-lipid complex (Eliasson 1994). However, the DSC method is a destructive method and can only provide the information of starch retrogradation, while the NIRS method

follows both physical and chemical changes in bread staling without any damage to the integrity of the bread.

Much research has focused on studying bread staling or amylopectin crystalinity in bread using NIRS (Wilson et al 1991; Osborne 1996, 1998; Xie et al in press). However, the research has provided only limited information about starch, protein, and temperature effects on bread staling. The primary objective of this study was to investigate the potential of NIRS as a fundamental tool to study bread staling with the help of a DSC. A second objective was to investigate starch, protein, and temperature effects on bread staling using NIRS and DSC.

3.3 Materials and Methods

3.3.1 Materials and bread preparation

Wheat starch, pre-gelatinized wheat starch (PREGEL N), and vital wheat gluten were obtained from Midwest Grain Products, Inc., Atchison, KS. Wheat starch contained a maximum of 0.3% protein and 9~12% moisture. The PREGEL N contained a maximum of 0.7% protein and a maximum of 10% moisture. Vital wheat gluten had a minimum of 75% protein, 5~8% moisture, 1~2% fat. Methyl cellulose (The Dow Chemical Company, Midland, Michigan) contained 85~99% methyl cellulose and 1~10% moisture. All the specifications were provided by the manufacturers.

Two kinds of bread samples were prepared for this study. The formulas for the starch-starch (SS) bread with 0% protein and starch-protein (SP) bread with approximately 15% protein are shown in Table 3.1. In the recipe for SP, 20% vital wheat gluten was added in order to obtain 15% protein in the formula. The recipes and bread making procedure were modified based on those published by Every et al (1998) and Morgan et al (1997).

Bread dry mix was obtained by mixing wheat starch, PREGEL N, methyl cellulose, vital wheat gluten, instant active dry yeast, and calcium propionate using a paddle in a standard 12 quart mixing bowl with a Hobart A-200 mixer (Hobart Corporation, Troy, OH) at speed one for 30 s. Sugar was dissolved in one third of the total water, added to the dry ingredients, and mixed at speed one for 30 s. Corn oil and the rest of the water were added and mixed at speed one for 30 s, then at speed two for 30 s. Finally, the dough was mixed at speed three for another 6 min for SS and 1.5 min for SP. The dough had a batter-like consistency. A total of 14 loaves of SS or SP dough of 210 g each was made from each batch. The dough was proofed in a pup loaf size pan at 40°C and 70~80% relative humidity (RH) for about 30 min or until the dough height was 2 cm over the pan. Dough was baked at 218°C for 26 min. Bread was kept on the shelf at room temperature and humidity for about 1.5 hour. Each loaf was cut into 12.5-mm-thick slices, wrapped into a single plastic bag, and stored in the incubator under one of the two storage conditions described below.

3.3.2 Experiment design and bread tests

A 2×2 treatment combination was used with a randomized complete block design in this experiment. Treatments were two protein levels, 0% and 15%, and two storage conditions, 1) 12.5∀1°C, 55∀3% RH, and 2) 31.5∀1°C, 88∀3% RH. Approximately 14 loaves of SS or SP were made from one batch on the same day for a block. Seven loaves of SS and seven loaves of SP were stored under one of the two incubator conditions. Five blocks (*a*, *b*, *c*, *d*, and *e* batch) were used in this experiment. Overall, approximately 140 loaves of bread were made.

In preliminary tests, the moisture losses for both the SS and SP bread stored at two different storage conditions were less than 1%. Specific loaf volume was measured by the rape seed displacement method. Moisture content was calculated by the following equation: MC = $\{(W_b\text{-}W_d)/W_b\}$ X 100%, where W_d is the total dry matter weight of flour and other ingredients, and W_b is the weight of bread loaf on the storage day (Wang and Sun 2002).

3.3.3 Data collection

A diode-array NIR spectrometer (Perten Instruments, Springfield, IL) was used to collect spectra. The NIRS specifications were reported by Xie et al (in press). One loaf of SS and one loaf of SP from each incubator were tested daily. Tests started approximately 24 hours after baking. Measurements were taken on all the slices, except two or three heel slices from each end in a loaf. The average of 15 scans taken on one slice in about one second were recorded as one spectrum.

About six NIRS spectra were taken from each loaf. The wavelength range of NIRS spectra was from 400nm to 1700nm.

After the NIRS test, two DSC tests were conducted on samples removed from a single loaf. DSC tests were done with a Perkin-Elmer DSC-Pyris1 instrument. Indium was used to calibrate the instrument. Samples were taken from the central portion of the central slice of each loaf using a cork borer (diameter 1.5cm) immediately after NIRS testing. The core was compressed and a ~45mg sample was sliced from the middle of the compressed core. The sample was put into a pre-weighed large volume aluminum DSC pan. The pan was hermetically sealed using a press. The pan and sample were then re-weighed. DSC scans were conducted from 5^oC to 130^oC at a 10^oC min^{-1} heating rate. An Al_2O_3 pan represented the reference scan.

3.3.4 Data analysis

Grams/32 software (Galactic, Salem, NH) and a partial least squares (PLS) regression (Martens and Næs 1989) were used to analyze NIRS spectra data. The raw spectra of LSS (SS at low temperature), LSP (SP at low temperature), HSS (SS at high temperature), and HSP (SP at high temperature) in batch *a* on day 1 are shown in Figure 3.1. All the raw spectra have a similar shape. Because of a high noise level below 550nm, the wavelength range from 550 to 1700nm was used for data analysis. Beta coefficients of PLS were used to determine the

important wavelength regions. The methods of developing calibration models were the same as described by Xie et al (in press).

The raw DSC thermograms of HSS and HSP in batch b on day 3 are shown in Figure 3.2. Two endotherm peaks were observed in a thermogram. Endotherm offset (T_o) and completion (T_m) temperatures were determined according to the method published by Wilson et al (1991). These two points were taken as the points at which deviation occurred from the linear portions of the traces before and after the endotherm. Endotherm enthalpies were computed in Joules per gram of dry matter of bread (J/g). The raw amylopectin retrogradation data were obtained by calculating the enthalpies of the first peaks in between 50^oC and 80^oC. The enthalpies of the second peak in between 105^oC and 120^oC were calculated as the raw amylose-lipid complex retrogradation data. The averaged raw amylopectin retrogradation value in one loaf was recorded as the DSC amylopectin retrogradation measurement of that loaf. The DSC amylose-lipid complex retrogradation measurements were obtained by following the same method.

The DSC amylopectin and amylose-lipid complex retrogradation measurements were correlated with NIRS spectra separately. In each validation batch, the NIRS-predicted amylopectin retrogradation data for all slices of one loaf were averaged and recorded as the NIRS amylopectin retrogradation measurements for that loaf.

3.4 Results and Discussion

DSC easily tracked starch retrogradation in bread. Amylopectin melting peak temperature ranged from $50^{o}C$ to $60^{o}C$ for bread stored at low temperature and from $60^{o}C$ to $70^{o}C$ for bread stored at high temperature. Figure 3.3 shows the amylopectin retrogradation development with time during bread storage in batch *a*. Other batches behaved similarly. The averaged correlation coefficient (R^2) of LSS, LSP, HSS, and HSP in all of the batches was 0.94, 0.93, 0.93, and 0.85, respectively. Amylopectin retrogradation trends of LSS and LSP fit better in logarithmic regression lines than in linear regression lines, while those of HSS and HSP fit better in linear regression lines. The sequence of all regression lines of all treatments was consistent, which was LSS, LSP, HSS, and HSP from the top to the bottom (Figure 3.3). This indicates that low temperature accelerated the amylopectin retrogradation process, especially during the early three days of storage. Linear regression was also applied to LSS and LSP data in order to compare the amylopectin retrogradation rates of all the treatments. The slopes of all linear regression lines of all treatments decreased in the following sequence: LSS, LSP, HSS, and HSP for five batches. In each batch, the slope of LSS was always higher than that of HSS and the slope of LSP was higher than that of HSP. This indicates that amylopectin at low temperature retrograded more rapidly than that at high temperature. Starch crystallization has a negative temperature coefficient in the temperature range $4^{o}C \sim 60^{o}C$ (Cornford et al 1964; Axford et al 1966; Slade and Levine 1987). High temperature retards bread staling by decreasing the starch retrogradation process.

It was also observed that the LSP regression line in each batch was always above the HSS line (Figure 3.3). Further, the slope of the LSP linear regression lines was higher than that of HSS. Even though LSP had 15% higher protein content than HSS, LSP staled faster than HSS. The results show that temperature dramatically affected the bread staling process. The effect of protein on bread staling was limited when compared to the temperature effect. At both temperatures, the regression line of SS was above that of SP (Figure 3.3). This indicates that protein slowed down the bread staling process. In addition, the slope of the SS linear regression line was always higher than that of SP at both temperatures. This shows that protein might retard bread staling not only by diluting starch as stated by Kim and D'Appolonia (1977a) and Every et al (1998), but also by interfering with amylopectin retrogradation. Erlander and Erlander (1969) reported that protein inhibited starch retrogradation by forming a complex with starch. The amide group of glutamine protein interacts with a glucose unit by a hydrogen bond in either the amylose or the amylopectin chain. The interaction between protein and starch will be discussed later.

NIRS spectra correlated strongly with DSC-measured amylopectin retrogradation data in each batch (Table 3.2). The range of R^2 was from 0.90 to 0.94 in the five batches. This indicates that NIRS was good at measuring amylopectin retrogradation in bread during storage. A calibration was developed by using samples in batches *d* and *e* because they had the highest and lowest averaged R^2. The calibration was used to predict retrograded amylopectin in batch *a*, *b*,

and *c*. Figure 3.4 shows the correlation of retrograded amylopectin as measured by NIRS and by DSC. For the combined three batches, the overall R^2 was 0.83 and SEE was 0.37. The results in each validation batch are also summarized in Table 3.2. The range of R^2 was from 0.90 to 0.96. The results show that NIRS could measure amylopectin retrogradation in other batches. The relative predictive determinate (RPD) is the ratio of standard deviation of reference data and standard error of estimation, which was used to evaluate the performance of the NIRS calibration (Williams and Norris 1987). RPD values for each batch ranged from 3.7 to 5.0, indicating that NIRS can measure amylopectin retrogradation precisely.

Figure 3.5 shows the beta-coefficients obtained from batches *a*, *b*, *c*, and calibration sample set batch *d* and *e* when three PLS factors were used. Three important wavelengths, 970nm, 1155nm, and 1395nm, are shown. The peak at 970nm might correspond to the second overtone of O-H and might be due to moisture and starch structure changes. The peak at 1155nm might be due to the second overtone of $C-H_3$. Finally, the peak at 1395nm might relate to the second overtone of C-H, $C-H_2$, and $C-H_3$. Moisture works as a plasticizer in the starch-gluten network when starch retrogrades. Moisture molecules must migrate into the crystal region while starch chain segments are realigning. Amylopectin crystallization leads to the development of a crystalline structure with a β-type crystalline region. At saturation, the β-type crystalline region has 27% moisture (w/w) (Imberty and Perz 1988; Slade and Levine 1991; Zobel and Kulp 1996).

This suggests that moisture migration contributed to the bread staling process. In fresh bread, amylopectin loses its crystallinity. During storage, amylopectin reforms into double helical structures and reorganizes into the crystalline region. When bread is reheated, amylopectin crystallinity is disrupted again (Zobel and Kulp 1996). The peaks at 1155nm and 1395nm might correspond to starch structure changes. The results from this study expanded the important wavelengths given by Wilson et al (1991) and Osborne (1998), which were 1414, 1465, and 1934nm.

NIRS-measured amylopectin retrogradation developments in validation batches *a*, *b*, and *c* were studied. NIRS-predicted amylopectin retrogradation data for all the slices in a loaf were averaged and recorded as the NIRS measurement for the loaf. The results in validation batch *b* are shown in Figure 3.6. The other two batches, *a* and *c*, behaved similarly. The sequence of regression lines of all treatments was the same as that obtained by DSC, except that HSS and HSP crossed around day 5 in batch *a*. The results indicate that NIRS could be used to study amylopectin retrogradation development in bread staling. It is reasonable to find out this since previous results show that NIRS spectra had a high correlation to DSC-measured amylopectin retrogradation. The results confirm the conclusion drawn by Wilson et al (1991) and Osborne (1998) that NIRS could follow the process of bread staling. They found that the staling rate constant for DSC data was consistent with that calculated from NIRS data. In addition, the results show that NIRS could study the staling process of samples having

extremely high or low protein contents and stored in various conditions. Previous research only investigated regular bread samples stored in room temperature and humidity. The R^2 obtained by NIRS was lower than that obtained by DSC, perhaps because of the small sample size. In a loaf, six to seven slices were available for NIRS scanning. On each test day, only one loaf was tested for one treatment. In future studies, 30 measurements per treatment on each test day would be recommended.

The amylose-lipid complex retrogradation, which Wilson et al (1991) and Osborne (1998) didn't study, was investigated using both DSC and NIRS in this study. Compared to amylopectin, the amylose-lipid complex had much less enthalpy and enthalpy change during storage. The amylose-lipid complex had an endothermic peak at 114±5°C, which was close to the value published by Eliasson (1994). Enthalpy values varied from 0.1 to 0.6 J/g for all treatments while that of amylopectin ranged from 0 to more than 3.0 J/g. This implies that the amylose-lipid complex contributed less to bread staling than amylopectin. Figure 3.7 shows the amylose-lipid complex development in batch *c*. The regression lines for all treatments were almost flat. Similar results were obtained in the other batches. The results indicate that the amylose-lipid complex didn't change as much as the amylopectin did while in storage. This confirmed that amylose changed little one day after baking. Amylose contributes to bread staling primarily during the first day of storage, but then changes little (Kim and D'Appolonia 1977b; Zobel and Kulp 1996). In all the five batches, SS showed

higher enthalpy values than SP, perhaps because less amylose was available in SP than in SS. The results agree with Kim and D'Appolonia (1977b). They noted that the effect of amylose on staling diminished as flour protein content increased because protein diluted starch.

NIRS spectra correlated poorly with the amylose-lipid complex retrogradation data measured by DSC. In batch b, the R^2 was 0.21 and 0.40 for SS and SP respectively. The results show that NIRS had difficulty in measuring the retrograded amylose-lipid complex in both SS and SP. Because of the small enthalpy values of the retrograded amylose-lipid complex, DSC measurements could be affected by many factors, such as lack of homogeneity in samples, uneven heating rates, and moisture distribution differences, etc. These factors also could cause difficulties for NIRS measurements. Even though NIRS didn't accurately measure the amylose-lipid complex retrogradation, PLS results show that NIRS could differentiate between SS and SP successfully. The beta coefficients showed two important wavelengths, 550nm and 1465nm, that helped NIRS differentiate SS and SP. The peak at 550nm showed that SS and SP had different colors. After baking, SS appeared whiter than SP because of the 15% protein in SP. The peak at 1465nm corresponded to the first overtone of N-H, which demonstrates that SS and SP had different protein contents.

NIRS has successfully been used in previous studies of bread staling, starch crystallinity, sucrose crystalline, and starch damage by following hydrogen bond

changes associated with O-H bonds in water and starch (Osborne and Douglas 1981; Davies and Miller 1988; Wilson et al 1991; Millar et al 1996; Osborne 1996). Gluten and starch can form a stable complex with "chelation-type" hydrogen bonds (Erlander and Erlander 1969). Hypothetically, this interaction could be detected by NIRS without any damage to the sample since it is a non-invasive method. Currently, no other analytical technique can investigate this interaction without sacrificing the integrity of the bread system. However, results from the current study show that this interaction was either too weak to be observed by NIRS or didn't play an important role in bread staling. In brief, this demonstrates that the starch and protein interaction was not as important as starch retrogradation in bread staling. This confirms the conclusion drawn by Every et al (1998) that gluten-starch interaction was less important than starch retrogradation due to the lower concentration of gluten. In addition, SS and SP had significantly different moisture content and specific volume, which might affect the results because the NIRS absorption value was used directly in data analysis.

3.5 Conclusions

NIRS could measure amylopectin retrogradation accurately in different batches. The averaged RPD value for NIRS measurements in validation batches was 4.3. NIRS and DSC obtained similar results when studying protein and temperature effects on amylopectin retrogradation development. However, NIRS had difficulty in measuring the changes of the amylose-lipid complex during storage. NIRS

spectra not only provided information about changes in bread moisture and starch structure, but also differences in bread color and protein content. Important wavelengths were 550nm, 970nm, 1155nm, 1395nm, and 1465nm. NIRS provides a useful approach to the study of the bread staling phenomenon. Bread staling is caused by changes in starch, protein, and moisture. Amylopectin retrogradation is likely the main factor in bread staling. The amylose-lipid complex contributes little to bread staling one day after baking. Temperature during storage significantly affects the amylopectin retrogradation process. Protein retards bread staling mainly by diluting starch. The starch and protein interaction reduces the staling rate, but is less important than starch retrogradation.

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Table 3.1 Formulas for the starch-starch (SS) and starch-protein (SP) bread.

Ingredients	SS (%)	SP(%)
Wheat starch	89.31	69.31
Pre-gelatinized wheat starch	9.95	9.95
Gluten	0.00	20.00
Methyl cellulose	0.74	0.74
	100%	100%
Sugar	5.48	5.48
Instant active dry yeast	1.98	1.98
Corn oil	3.29	3.29
Water	105.95	105.95
Mold inhibitor(calcium propionate)	0.22	0.22

Note: All of the data reported in this table are based on flour = 100%.

Table 3.2 Summary of visible near-infrared reflectance spectroscopy (NIRS)

measured retrograded amylopectin results.

Batches	Cross-validation			Validation [a]		
	No. Factors	$R^{2\,b}$	SECV [c]	R^2	SEE [c]	RPD [d]
a	10	0.91	0.28	0.90	0.25	3.7
b	10	0.94	0.22	0.96	0.21	4.2
c	10	0.93	0.23	0.94	0.18	5.0
d	11	0.94	0.26	~	~	~
e	9	0.90	0.28	~	~	~
Average	~	0.92	0.25	0.91	0.21	4.3

[a] A calibration was developed from batch *d* and *e* (factors = 9) and used to measure staling of samples in *a*, *b*, and *c* batches.
[b] R^2 is with differential scanning calorimetry (DSC) measured retrograded amylopectin.
[c] SECV = standard error of cross-validation, and SEE = standard error of estimation.
[d] RPD = Relative predictive determinate.

The sequence of absorption value at the peak:
HSS>LSS>HSP>LSP
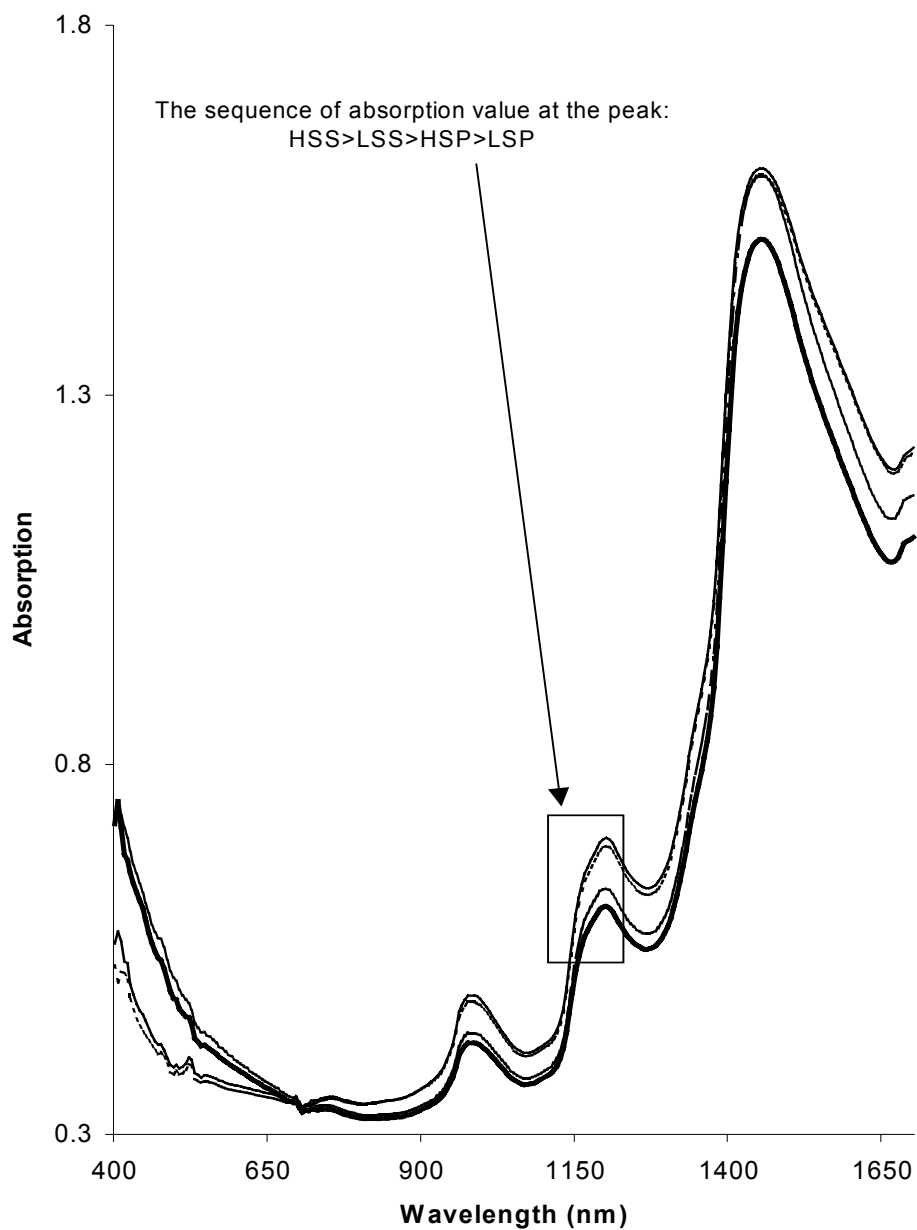
Figure 3.1 Raw spectra of the starch-starch bread stored at low

temperature (LSS) and high temperature (HSS), and the starch-protein

bread stored at low temperature (LSP) and high temperature (HSP) in batch

***a* on day 1.**
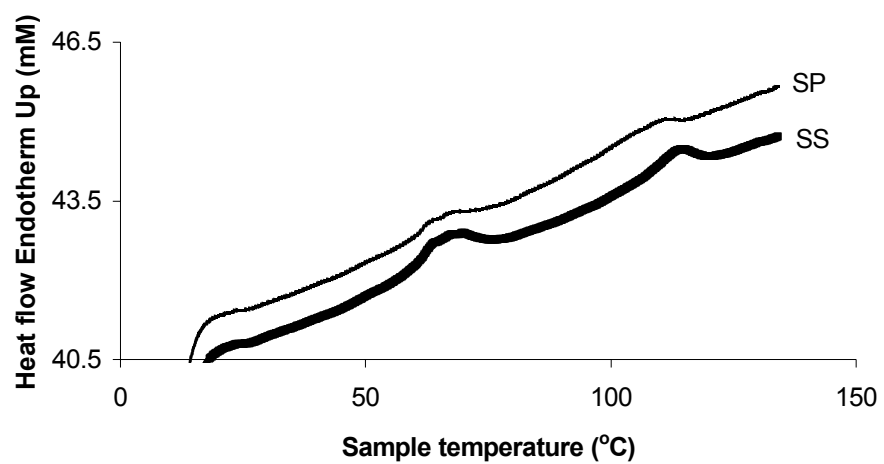
Figure 3.2 Raw DSC thermogram of the starch-starch (SS) and starch-protein (SP) bread in batch *b* stored at high temperature on day 3.
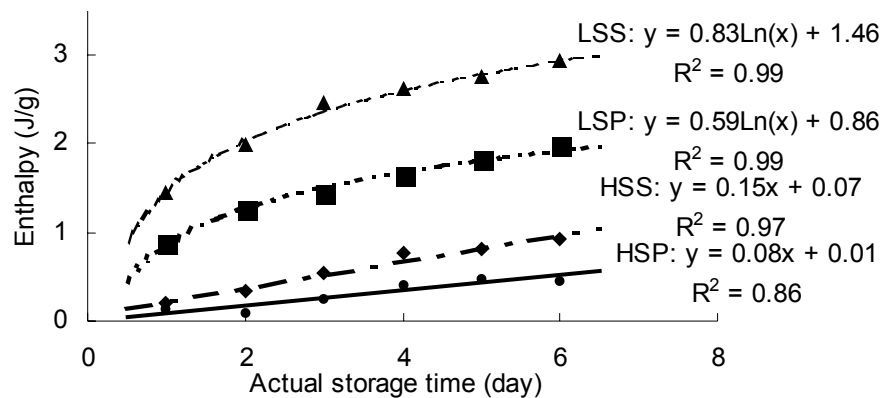
Figure 3.3 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by differential scanning calorimetry (DSC) in batch *a*.
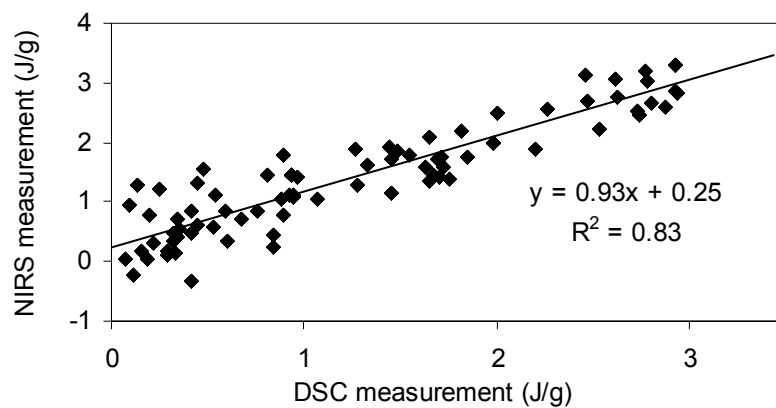
Figure 3.4 The correlation of retrograded amylopectin measured by visible and near-infrared spectroscopy (NIRS) with that by differential scanning calorimetry (DSC) in validation batch *a, b*, and *c*.
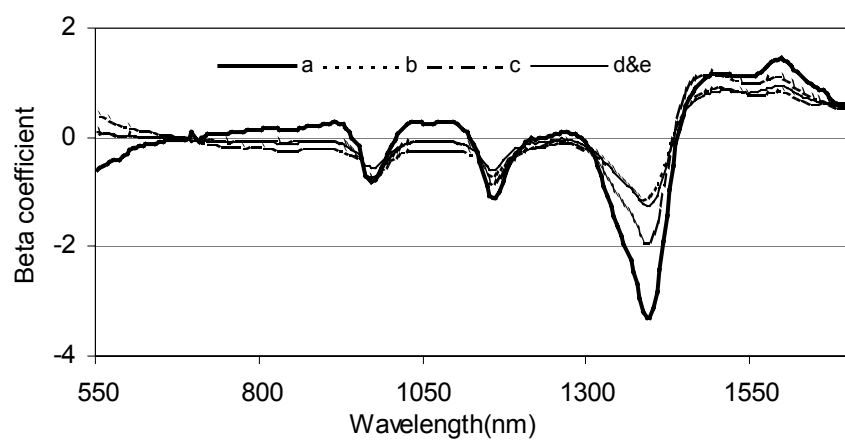
Figure 3.5 Important wavelengths for amylopectin retrogradation from calibration batch d and e and validation batch *a*, *b*, and *c*.
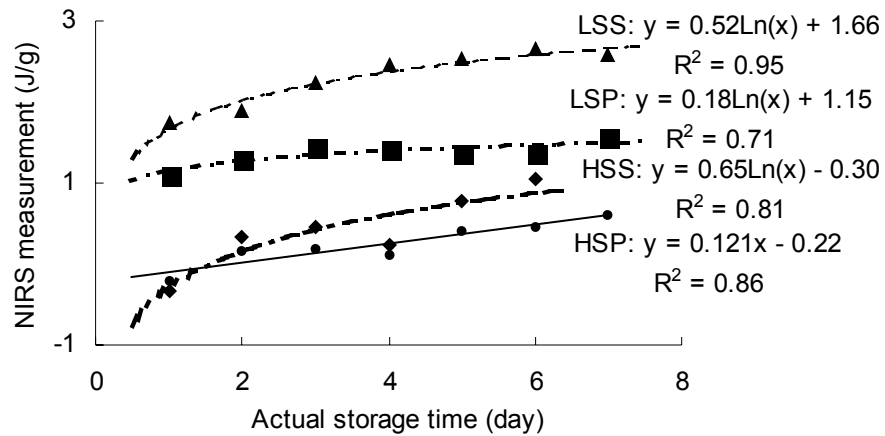
Figure 3.6 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by visible and near-infrared spectroscopy (NIRS) in batch _b_.
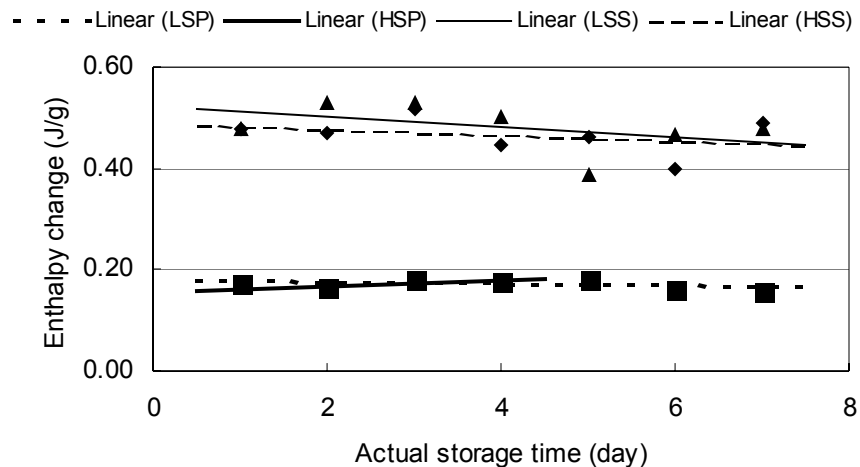
Figure 3.7 The amylose-lipid complex retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch *c*.

4. SUMMARY AND FUTURE RESEARCH

4.1 Summary

The potential of NIRS as a fundamental tool for studying bread staling was investigated in this research. How well NIRS can measure bread staling was studied first by comparing NIRS results with those obtained by the TA. TA measures bread firmness and is a common method to evaluate bread staling. Twenty-five loaves of commercial wheat white pan bread from one batch were studied over 5 days and five different batches were used. NIRS spectra had a high correlation to TA firmness. Compared to TA firmness, NIRS measurements had higher correlation to the actual storage time and a smaller standard deviation. The effect of the batch differences on NIRS measurements was not as much as that on TA firmness. Results indicate that NIRS could monitor changes during bread storage more precisely than TA. The potential of NIRS was further investigated by studying starch, protein, and temperature effects on bread staling using a DSC. DSC is commonly used to measure starch retrogradation. A 2×2 treatment combination was used with a randomized complete block design in this experiment. Approximately 14 loaves of SS or SP were made from one batch (block) and five batches were used. Five important wavelengths, 550nm, 970nm, 1155nm, 1395nm, and 1465nm, show that that NIRS could be a useful tool to study bread staling. These wavelengths also indicate that NIRS monitored changes in starch, protein, and moisture. All the changes in starch, protein, and moisture contributed to bread staling. Amylopectin retrogradation could largely account for bread staling. The amylose-lipid complex contributed little to bread

staling after one day of storage. Temperature had a significant effect on the bread staling process. The interaction between starch and protein was not as important as starch retrogradation. Protein retarded bread staling by diluting starch and reducing starch retrogradation. The result supports the hypothesis proposed above that bread staling is due to changes in starch, gluten, and moisture. A combination of the theories proposed by Schiraldi et al (1996), Zobel and Kulp (1996), and Every et al (1998b) is more reasonable.

4.2 Future research

To further investigate the potential of NIRS as a fundamental tool for studying the bread staling phenomenon, future research should investigate specific volume effects on NIRS measurements. It is more practical to scan bread crust instead of crumb when applying NIRS to measure bread freshness. Therefore, changing the scanning method of NIRS will be more useful for its future applications. Future research should study this. Future research also needs to study the staling phenomenon in different bread types. The NIRS method may be also applied to study the effect of anti-staling agent in the future research. Theoretically, hydrogen bonds between gluten and starch could be detected by NIRS. Future research may repeat this study using samples having more constant moisture content and specific volume. To use a NIRS system with a wider wavelength range is also a good choice.

5. APPENDIX.
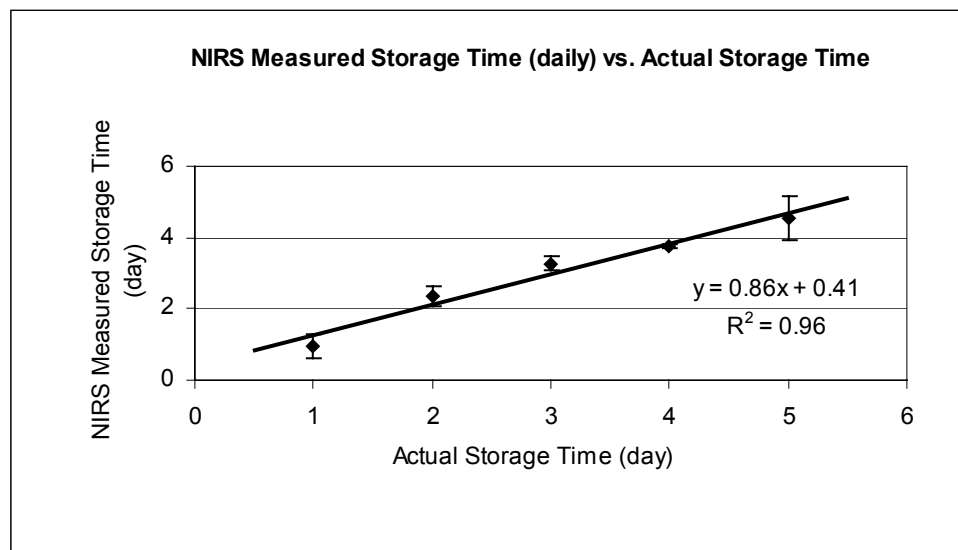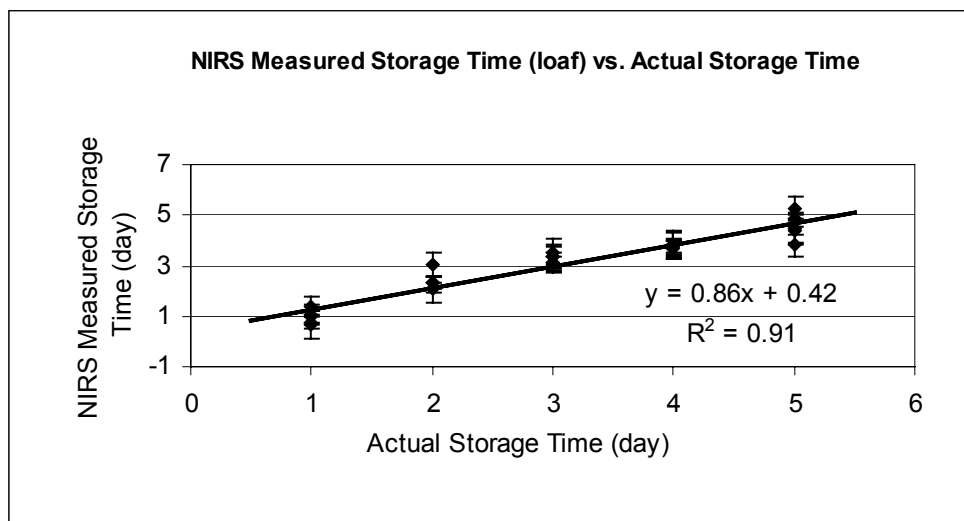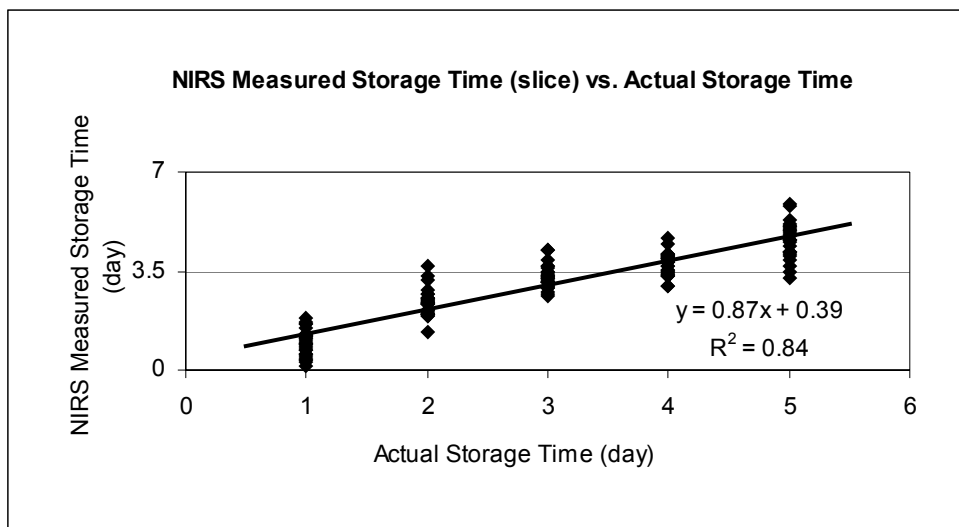
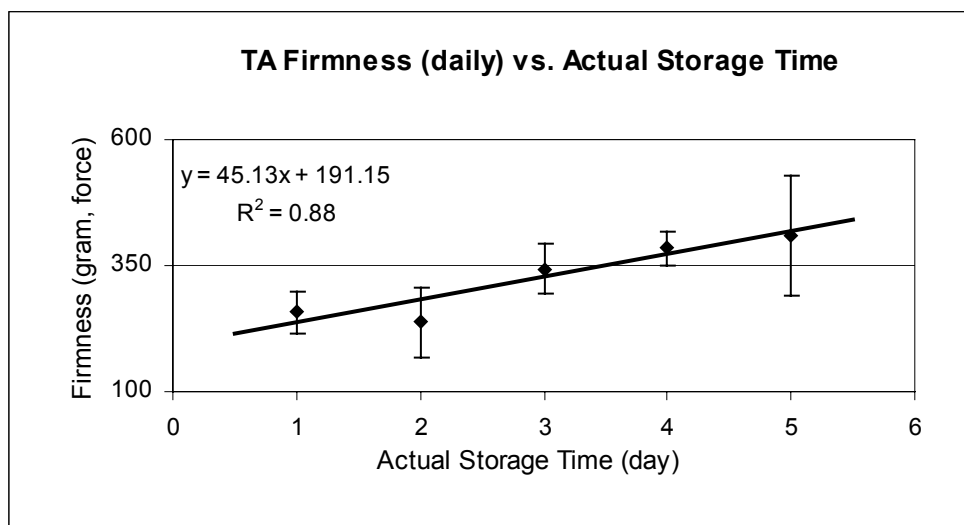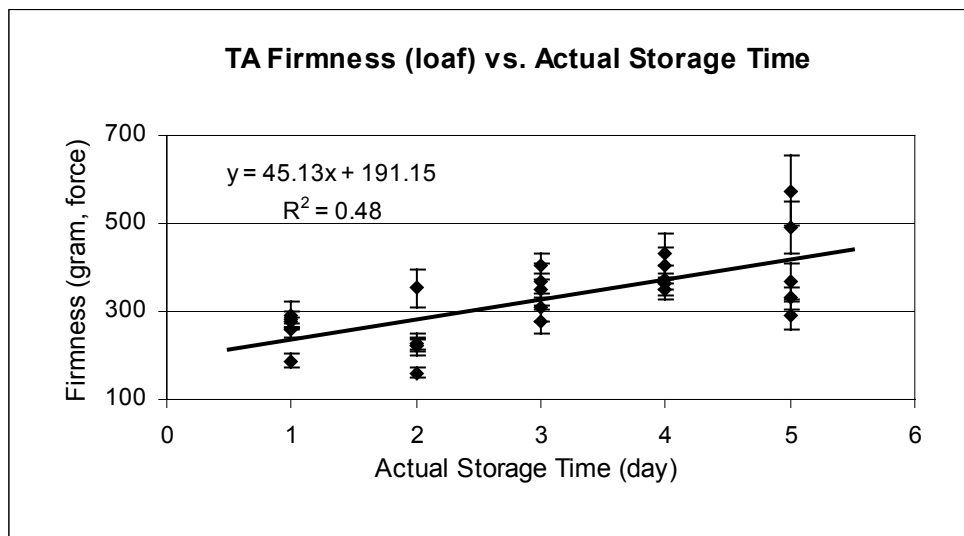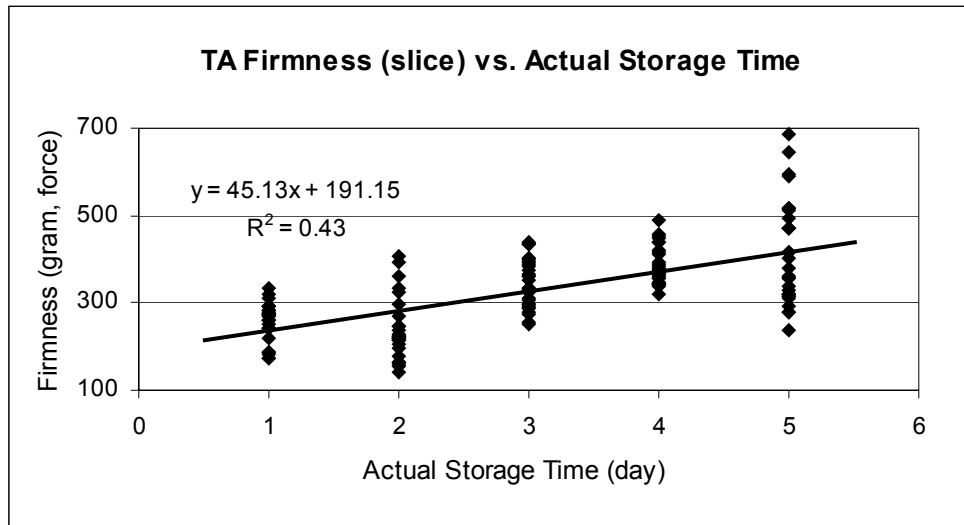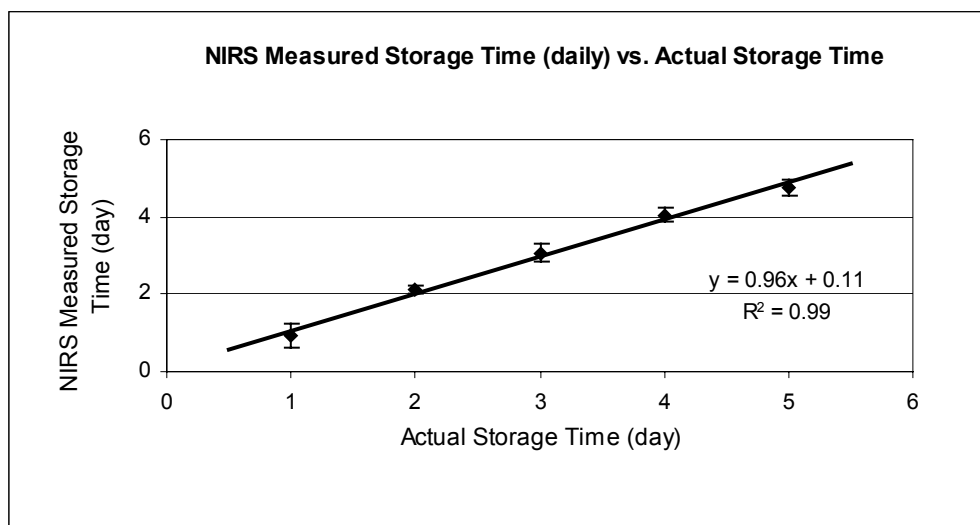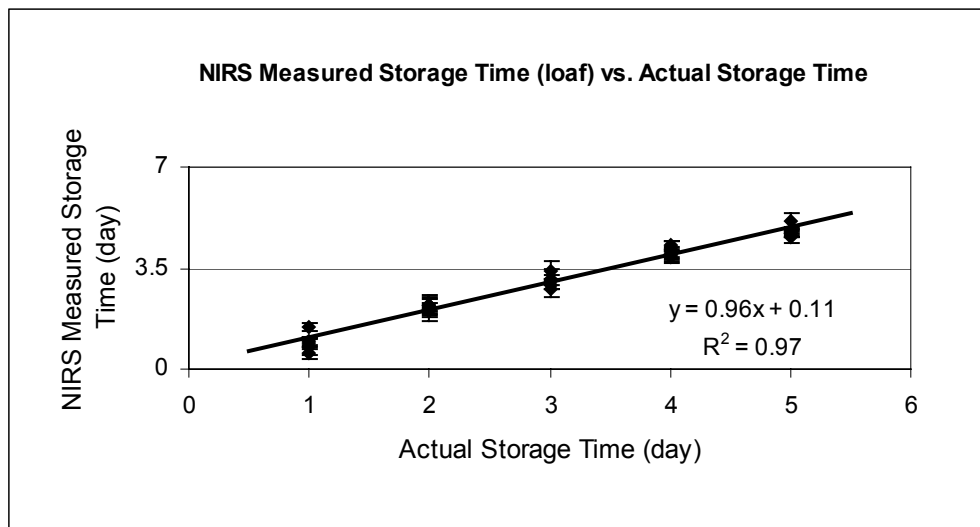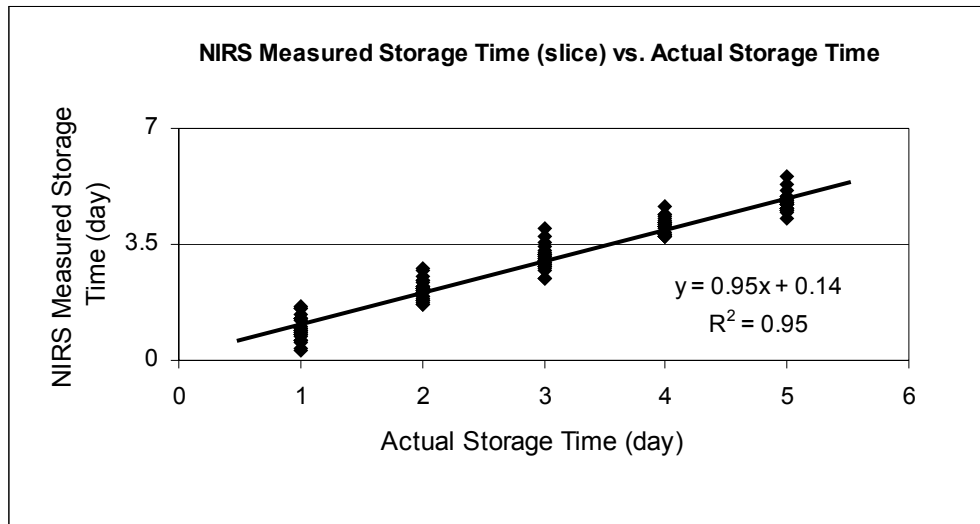**Figure 5.1 NIRS cross-validation results in batch _a_.**
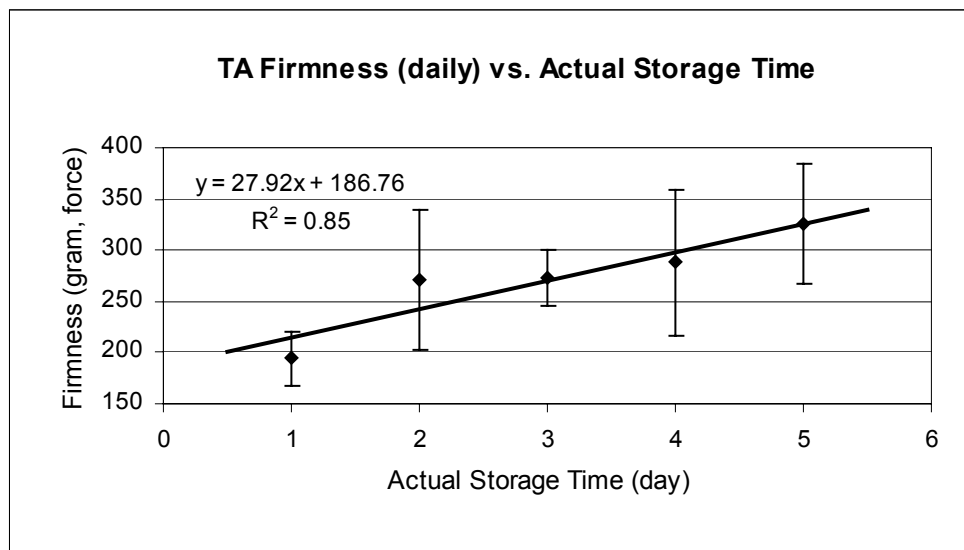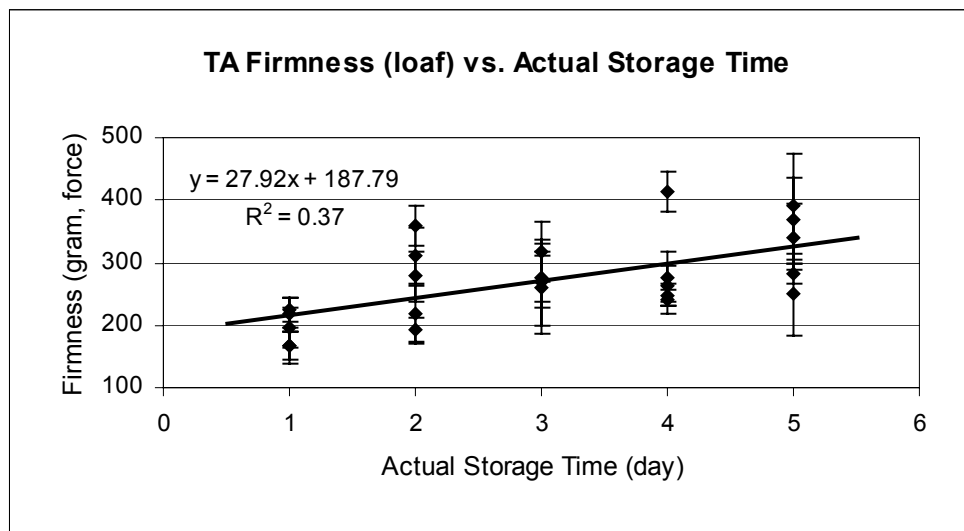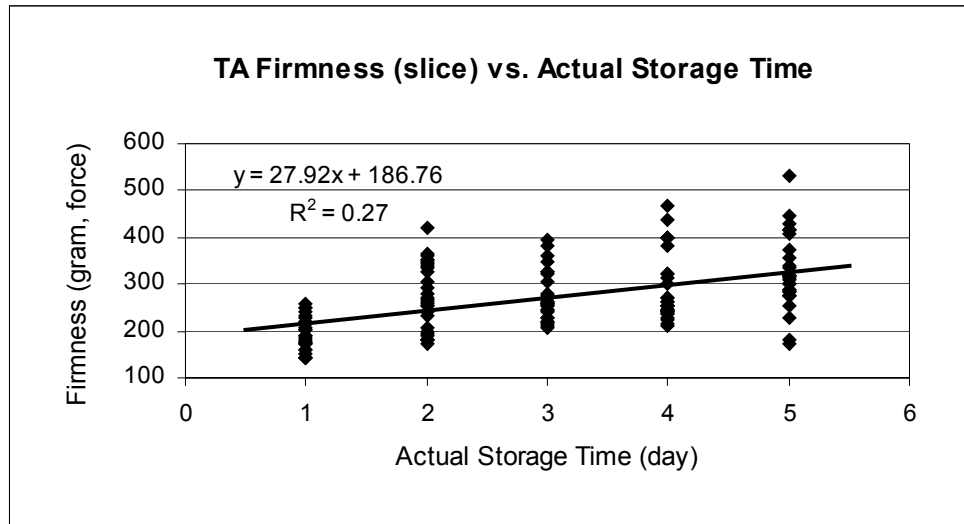
Figure 5.2 Correlation of TA firmness with actual storage time in batch *a*.
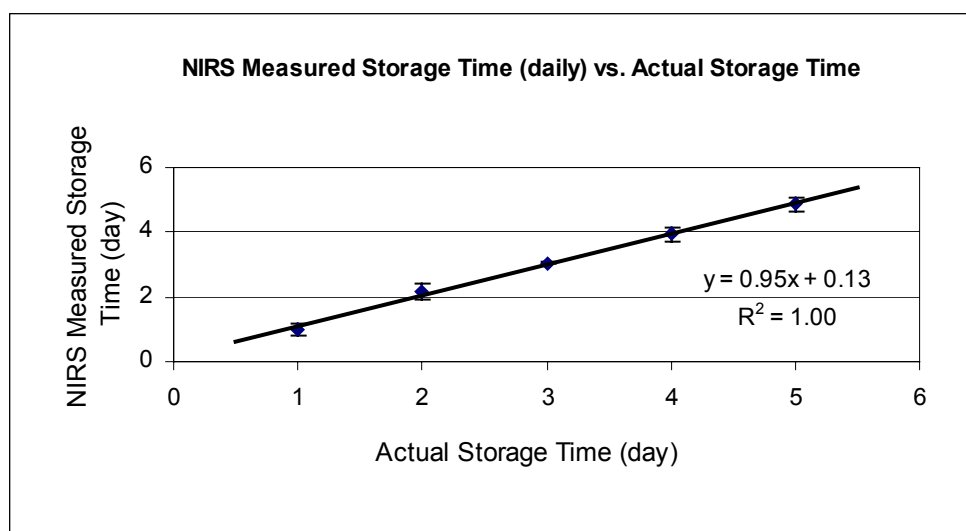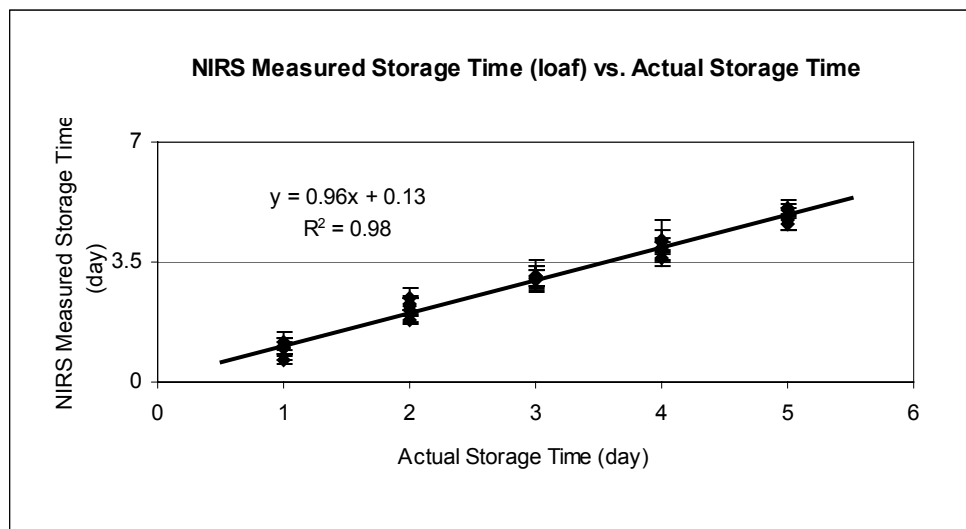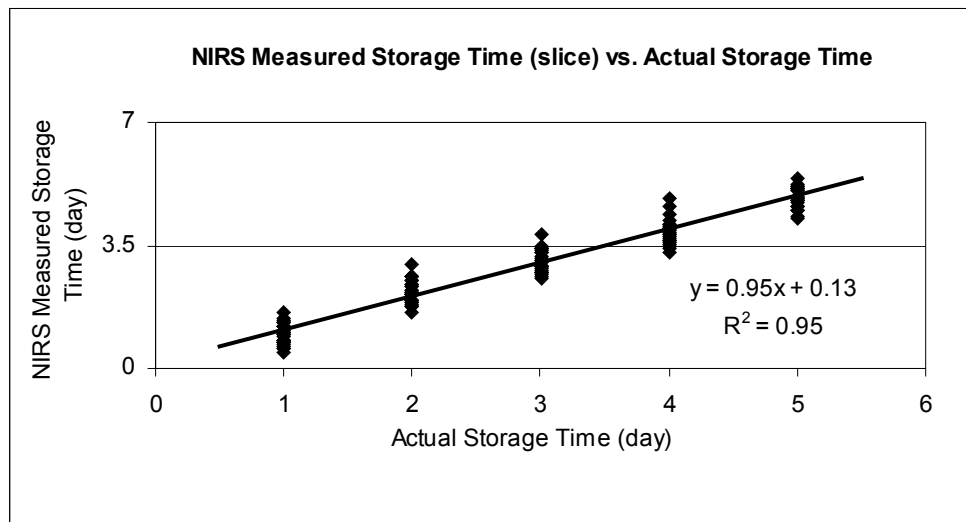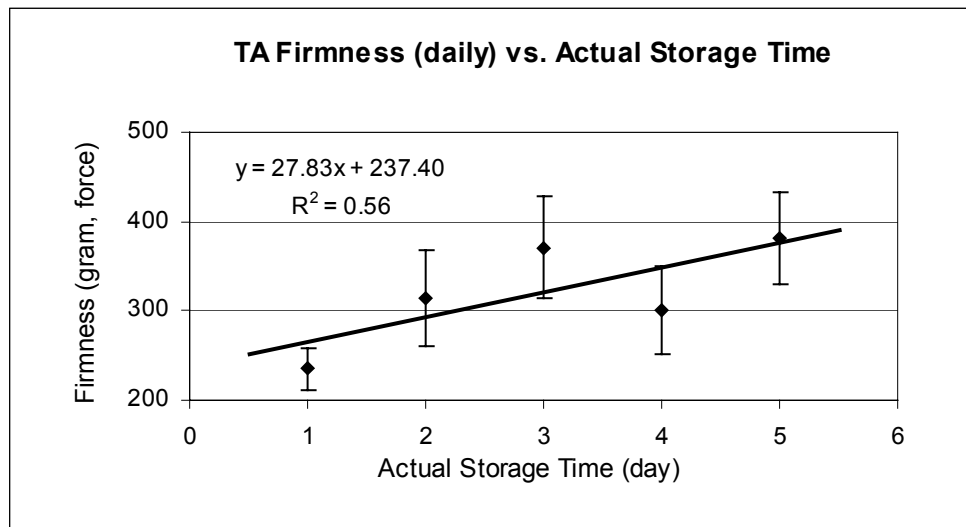
Figure 5.3 NIRS cross-validation results in batch *b*.
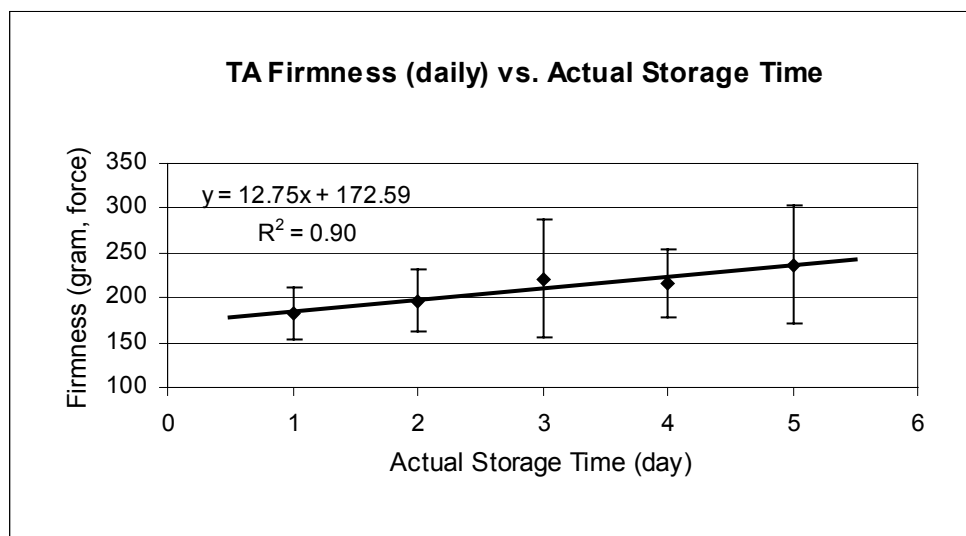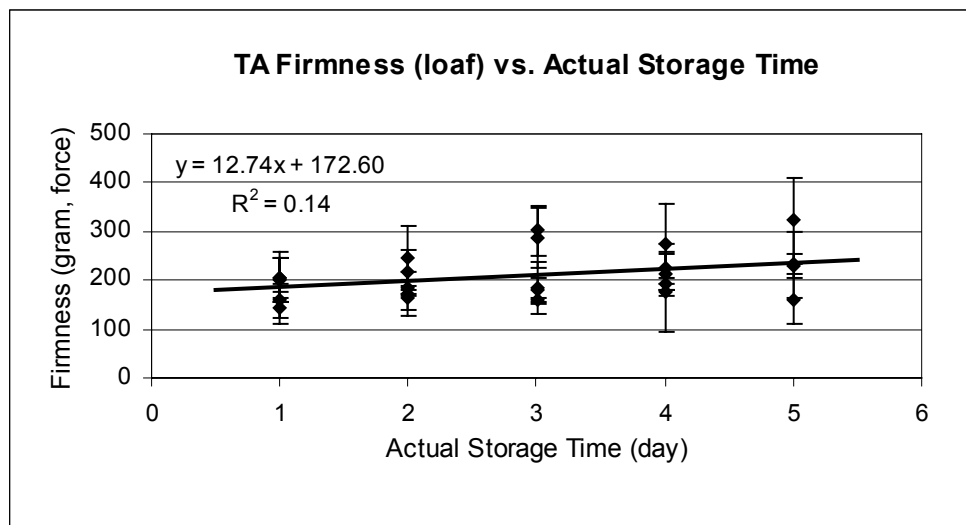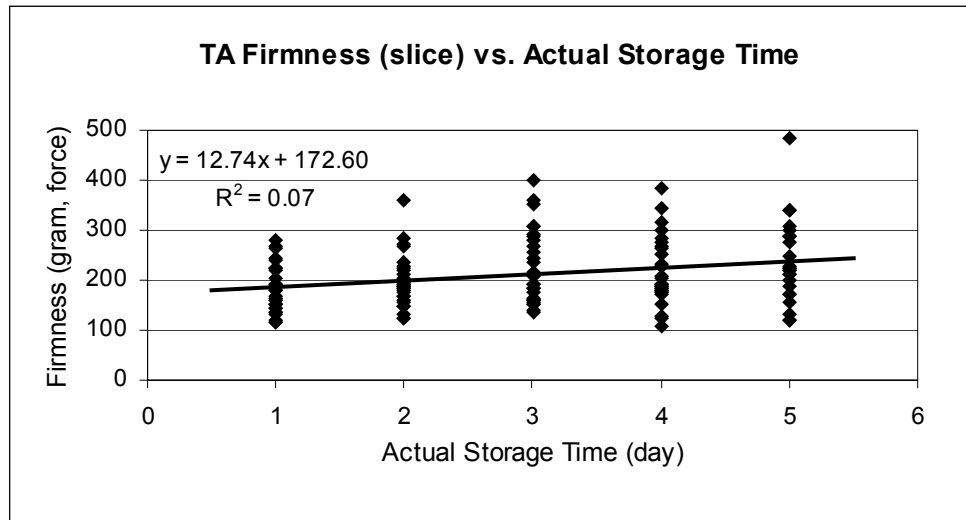
Figure 5.4 Correlation of TA firmness with actual storage time in batch *b*.
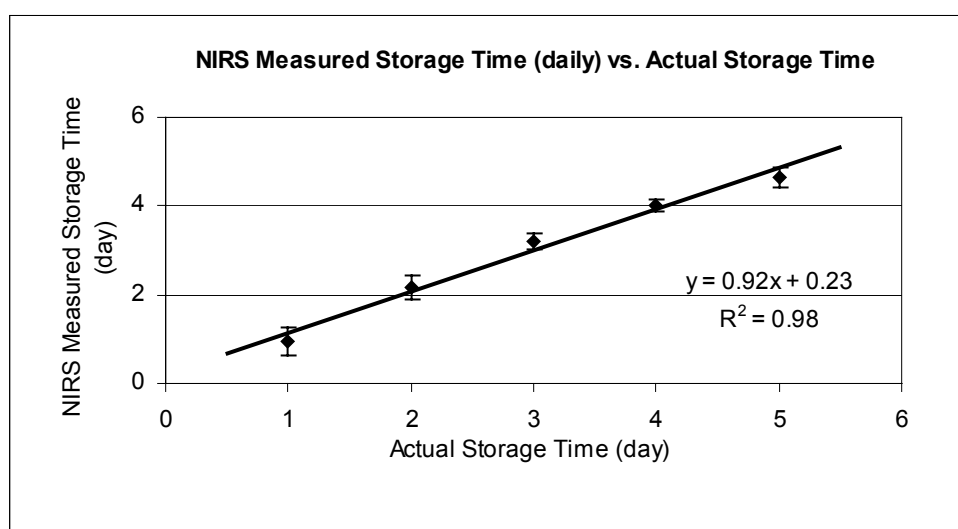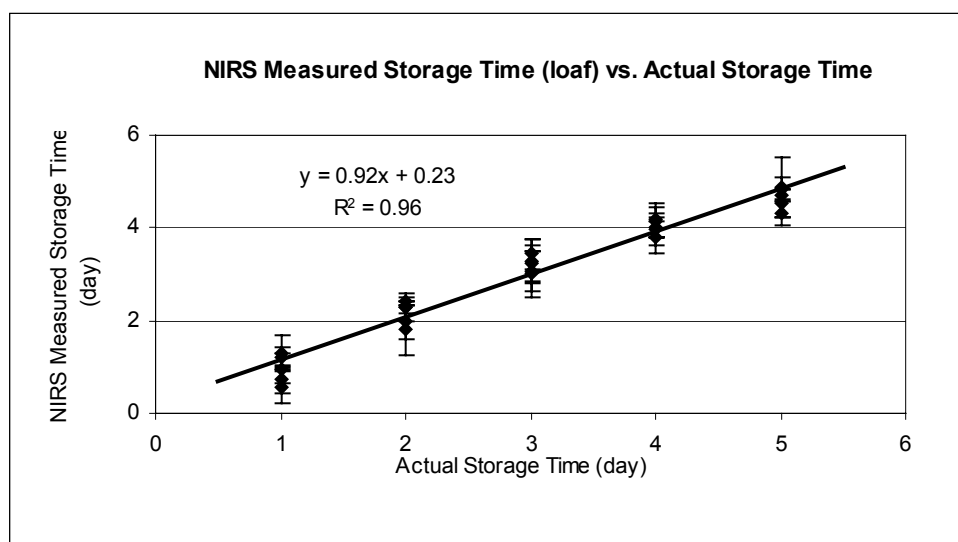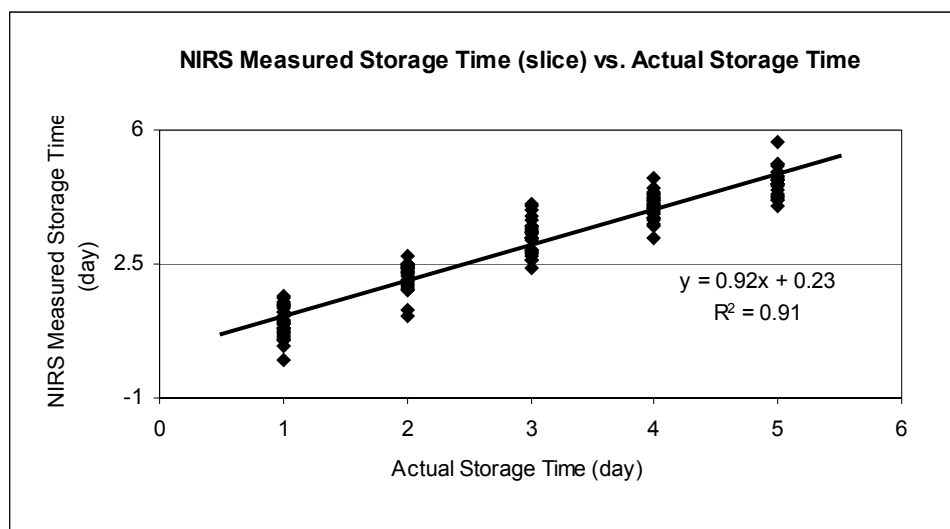
Figure 5.5 NIRS cross-validation results in batch _d_.

Figure 5.6 Correlation of TA firmness with actual storage time in batch *d*.

Figure 5.7 NIRS cross-validation results in batch e.
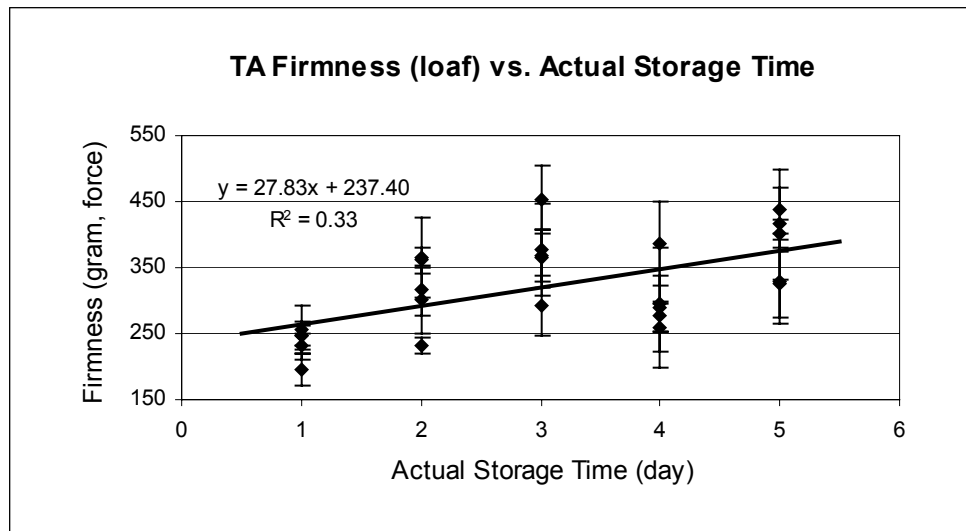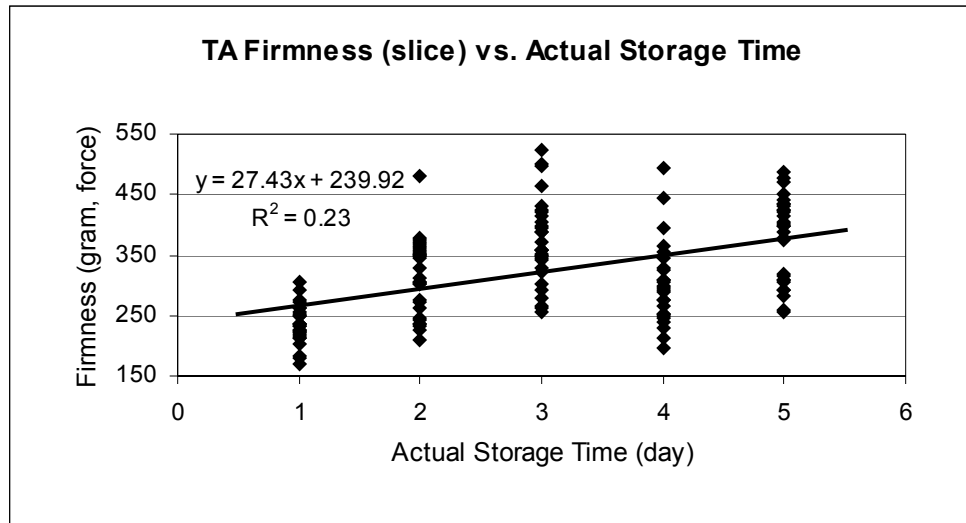
Figure 5.8 Correlation of TA firmness with actual storage time in batch e.
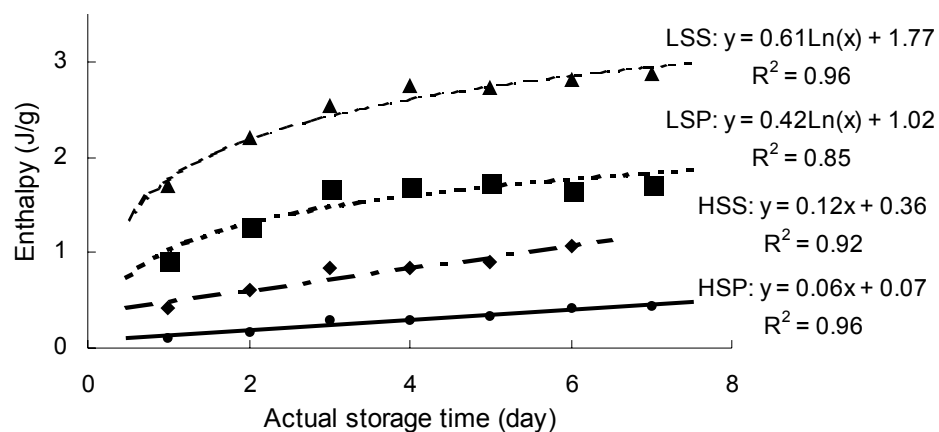
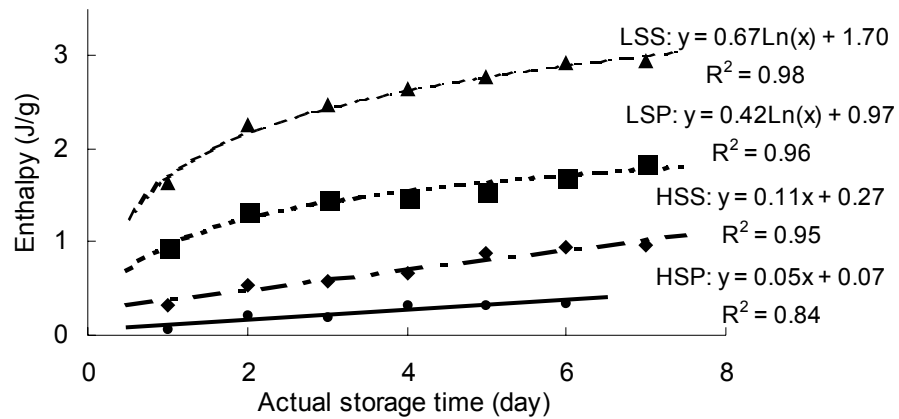**Figure 5.9 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by differential scanning calorimetry (DSC) in batch _b_.**

The graph contains the following labels and equations:

LSS: y = 0.67Ln(x) + 1.70
R^2 = 0.98
LSP: y = 0.42Ln(x) + 0.97
R^2 = 0.96
HSS: y = 0.11x + 0.27
R^2 = 0.95
HSP: y = 0.05x + 0.07
R^2 = 0.84

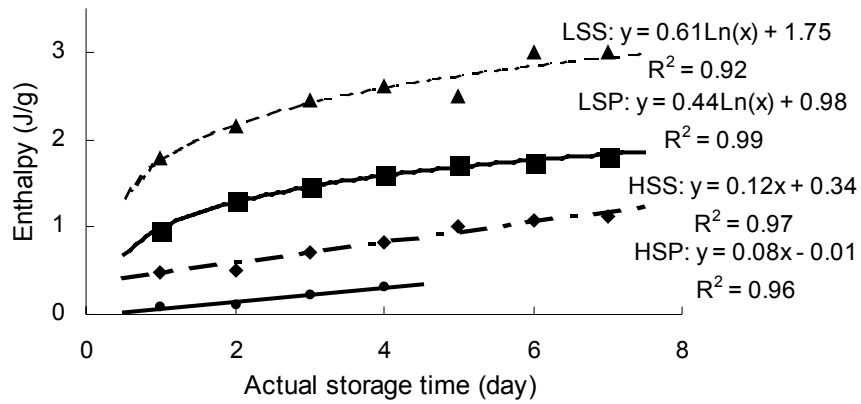Enthalpy (J/g)

Actual storage time (day)

Figure 5.10 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by differential scanning calorimetry (DSC) in batch *c*.
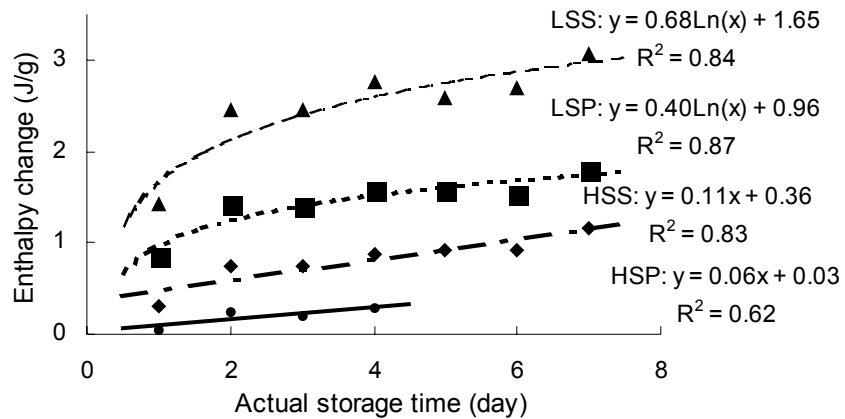
Figure 5.11 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by differential scanning calorimetry (DSC) in batch *d*.
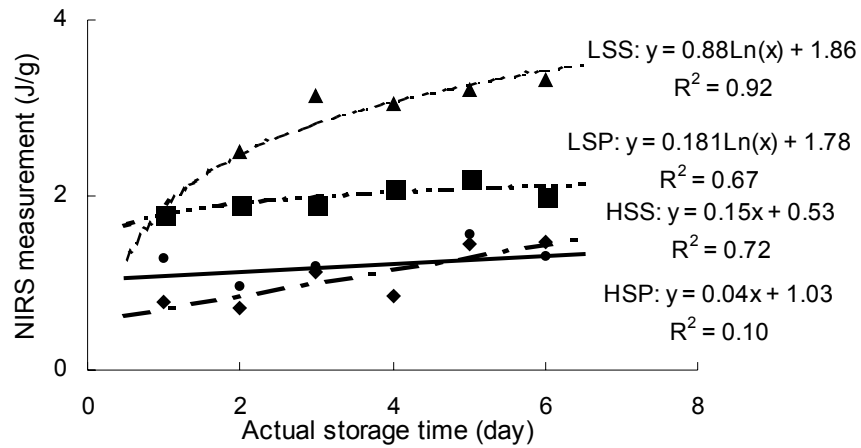
Figure 5.12 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by differential scanning calorimetry (DSC) in batch e.
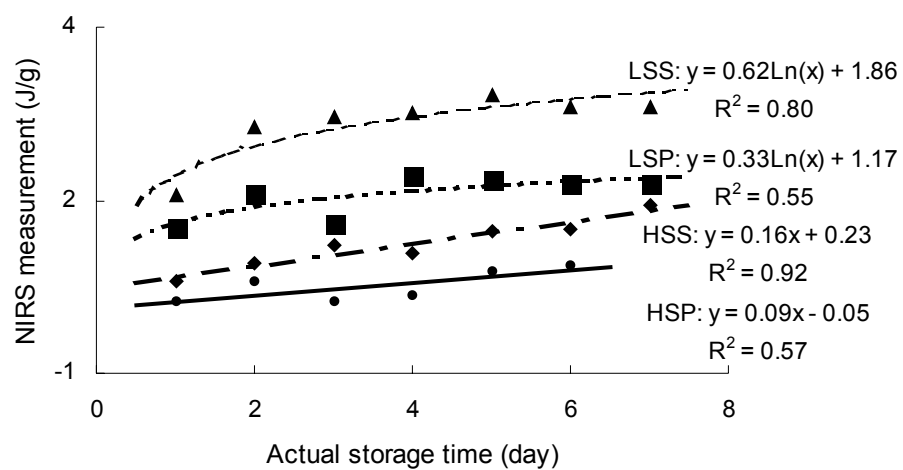
Figure 5.13 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by visible and near-infrared spectroscopy (NIRS) in batch *a*.

Figure 5.14 Amylopectin retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) as followed by visible and near-infrared spectroscopy (NIRS) in batch *c*.
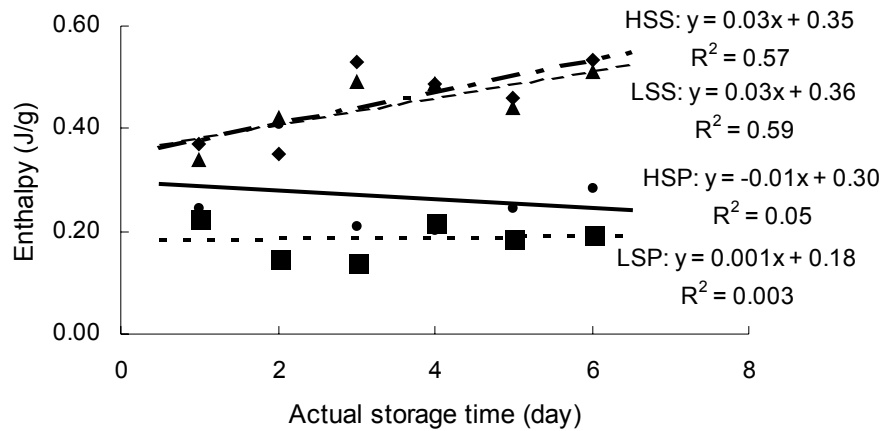
Figure 5.15 The amylose-lipid complex retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch *a*.

HSS: y = -0.002x + 0.51

$R^2 = 0.003$

LSS: y= -0.013x + 0.56

$R^2 = 0.43$

HSP: y = 0.01x + 0.16

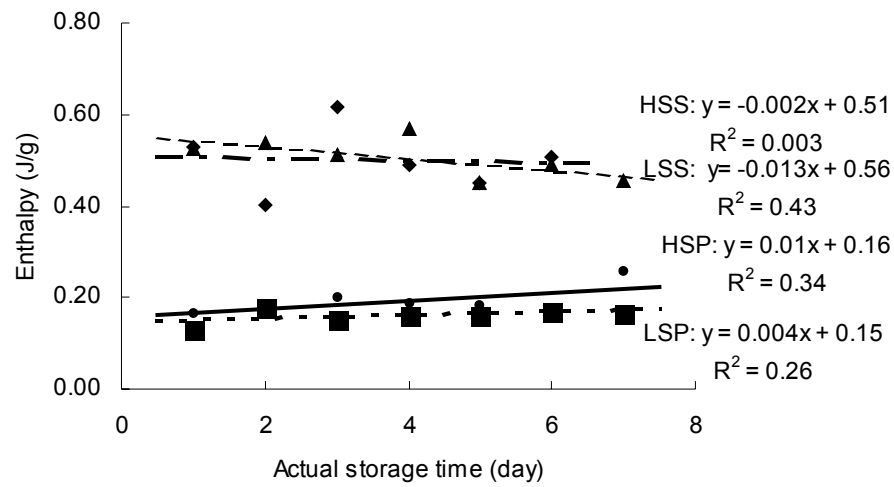$R^2 = 0.34$

LSP: y = 0.004x + 0.15

$R^2 = 0.26$

Figure 5.16 The amylose-lipid complex retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch *b*.
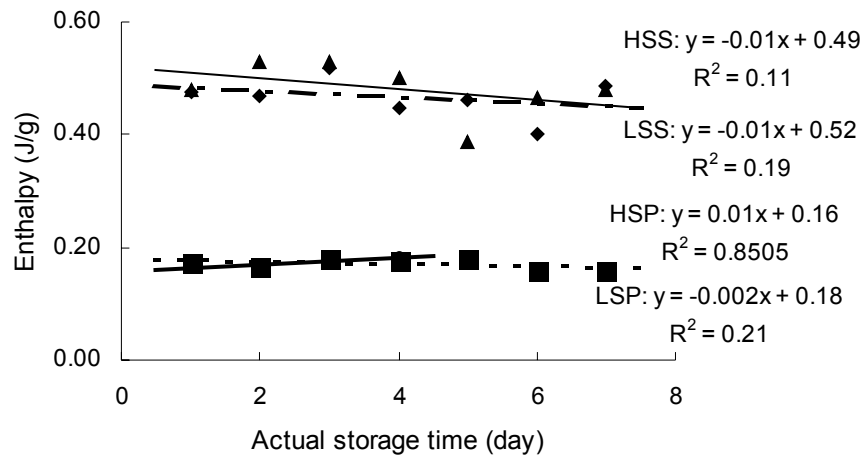
Figure 5.17 The amylose-lipid complex retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch *d*.
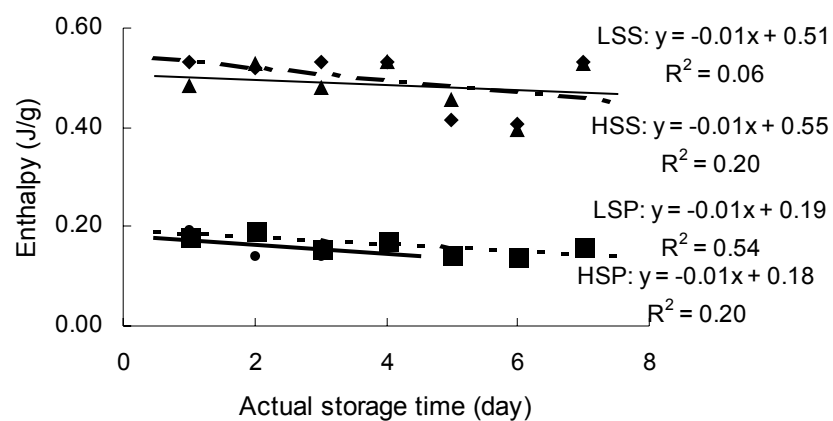
Figure 5.18 The amylose-lipid complex retrogradation trend of the starch-starch bread stored at low temperature (LSS) and high temperature (HSS), and the starch-protein bread stored at low temperature (LSP) and high temperature (HSP) measured by differential scanning calorimetry (DSC) in batch e.